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RELATED TO HIGHER CATEGORY SYSTEMATICS
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MALACOLOGIA, 1984, 25(1): 1

FUNCTIONAL MORPHOLOGY AND ONTOGENY OF MOLLUSCA AS RELATED TO HIGHER CATEGORY SYSTEMATICS: INTRODUCTION

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The fields of evolutionary biology and systematics are timely topics among biologists today. In many journals and books new interpretations of the process of evolution and reevaluation of the methodology employed in systematic analyses are discussed. There is, however, a tendency to formulate new hypotheses and theories that are based on poor data derived from unsound, older taxonomic work. Some workers appear to eschew the frequently long and meticulous descriptive work that is necessary, often at the alpha level, to interpret morphological patterns and derive any meaningful analyses of higher taxa. Sound descriptive work uses anatomical and hard part analyses, and the study of ontogeny and adaptive functional morphology. These provide the characters forming the basic data necessary for complete analysis of taxa. Without these kinds of data innovative systematic approaches are impossible whether they be phenetic, cladistic or traditional.

In even the most common of molluscan groups detailed descriptive work that includes anatomy is surprisingly incomplete or lacking. Many higher taxonomic categories are poorly defined and based solely on variable shell characters. These characters are frequently deficient as discriminant tools even at the specific level; yet, new supraspecific taxa are constantly being proposed and accumulate at a rate which leads one to question their validity and usefulness. Perusal of the latest *Zoological Record* revealed that 116 Recent molluscan higher taxa were proposed in one year. It is doubtful that many of these taxa have their salient characters well-defined and quantified or represent any major adaptive significance. Clearly, there is a real need for careful, holistic descriptive work. The goal of this symposium was to demonstrate that data derived from the study of the functional morphology and ontogeny of molluscan groups can be applied in the formulation of valid, well-defined, higher taxonomic categories. These, in turn, allow malacologists to make meaningful comparisons between groups. The resulting taxonomic judgements and established polarities can then be developed into testable hypotheses about the systematic relationships of mollusks.

This symposium complements the 1977 AMU Symposium on the Evolution and Adaptive Radiation of Mollusca. At that time, editor Davis remarked that the majority of papers dealt with land snails because most of the recent work on molluscan evolution has involved terrestrial snails. In general, anatomical studies have been more common on land and freshwater taxa than among the marine groups. It was thus gratifying to see that malacologists are now beginning to work on the anatomy and ontogeny of some of the vast number of marine groups which are known only by their shells. In the 1981 symposium, fourteen papers were presented covering terrestrial, freshwater, and marine groups. Of these, six were on marine groups, four on freshwater taxa and two on land snails. Two papers given by Linsley and Yochelson, respectively, dealt with Paleozoic taxa, showing what can be done by careful analysis of hard parts. Waller's paper demonstrated what can be derived from an ontological study of shell characters in elucidating the function of convergent structures. The McLean and Hickman papers approached the relationships of extinct taxa to Recent ones by using data derived from functional morphological analyses of living species. A paper by F. C. Thompson has been added to the Symposium Proceedings because it forms a unit with the papers of Davis and Ponder on rissoacean systematics. The paper given by Warén was withdrawn from publication at his request because he felt that it was too incomplete to appear in print at this time.

MALACOLOGIA, 1984, 25(1): 3–20

A CASE FOR DERIVATION OF THE FISSURELLIDAE FROM THE BELLEROPHONTACEA

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ABSTRACT

Golikov & Starobogatov's (1975) theory that the Paleozoic bellerophontaceans gave rise to the Mesozoic-Recent Fissurellidae is considered. Just as the Haliotidae are limpet descendants of the Pleurotomariidae, the Fissurellidae are the logical limpet derivatives of the Bellerophontacea. Both derivations require suppression of coiling, shortening of the mantle cavity, and the addition of afferent ctenidial membranes.

Arguments favoring this derivation are treated: 1) Fissurellid anatomy differs in major ways from that of other dibranchiate gastropods, particularly the nearly vestigial left kidney. This anatomy could have been shared with the coiled predecessor, the bellerophontaceans. 2) Shell structure of crossed-lamellar aragonite shared by fissurellids and bellerophontaceans supports common ancestry. 3) Contrary to previous accounts, there is no several-whorled, orthostrophically coiled early phase in fissurellid ontogeny. Suppression of bellerophontacean coiling allowed the inherent asymmetry of torsion to have an immediate effect, producing the varying degrees of postprotoconch asymmetry expressed in the Fissurellidae. 4) The fissurellid postprotoconch shows whorl overlap (as does the mature bellerophontacean), brought about by delayed development of the columellar lip. 5) The internally directed, hook-shaped process of the fissurellid muscle scar may be homologous with the "oblique transdorsal element" of the bellerophontacean muscle scar. 6) Morphology of apertural slits in fissurellids and bellerophontaceans is similar. 7) The mantle edge in fissurellids can envelop the shell without obliterating the surface sculpture. The apertural shape of bellerophontaceans suggests that some had partially to completely internal shells; mantle folds like those of fissurellids would have allowed elaborate sculpture to be retained on internal shells.

Although intermediate forms have not been found as fossils, the evidence favors this derivation. The origin of fissurellids would have been a rapid, paedomorphic event. There are no alternative hypotheses for the origin of fissurellids. If fissurellids were derived from bellerophontaceans, the late Paleozoic members, at least, were torted gastropods.

INTRODUCTION

Although the limpet form is represented in many diverse families of gastropods, all are thought to have been derived from coiled predecessors. I have previously discussed the origin of limpets (McLean, 1981), noting that there are so many diverse anatomies represented in limpet families that it is apparent that the form itself imposes few constraints upon the internal anatomy. The essential features of the anatomy of a limpet should therefore be similar to those of its coiled predecessor.

The Fissurellidae, which first appeared in the Mesozoic, are dibranchiate limpets in which the adult shell and mantle cavity organs, particularly the ctenidia, are essentially bilaterally symmetrical; in contrast, other families with paired gills are markedly asymmetrical. Because there is some indication of asymmetrical coiling in the early stages of fissurellids, such authors as Yonge (1947) and Eales (1950) have assumed that the bilateral symmetry of the adult is secondary and that they have therefore been derived from asymmetrically coiled predecessors. Currently, the Fissurellidae are placed in the suborder Pleurotomariina (Cox & Knight, 1960; Knight *et al.*, 1960). These authors offered no theory of fissurellid origin, nor did Batten (1975: 26), who concluded: "Because the Fissurellidae have few shared derived features with any pleurotomariacean group, no likely ancestors are now known."

Golikov & Starobogatov (1975: 198), offered an intriguing theory, claiming that the Fissurellidae were derived from the Paleozoic Bellerophontacea:

> We think it reasonable to unite in one group the planispiral Bellerophontoidea, which became extinct in the Triassic, and the limpet-shaped Fissurelloidea, which appeared at that time, in particular if we take into account that the development of the latter group does not show any trace of a conispiral shell in their ancestors. Besides the presence in Fissurelloidea of a cap-like shell with a horseshoe-shaped columellar muscle developing from nearly equal rudiments (Crofts, 1955), its usual symmetry, and similarities in the location and size of the mantle complex together with various progressive features in the structure of the nervous system, provide good reason to consider this group as derived from Bellerophontoidea, which had a planispiral endogastric shell.

Golikov & Starobogatov gave no further discussion; their assignment of the Fissurellacea and the Bellerophontacea to an order Dicranobranchia Gray, 1821, has been ignored by subsequent authors.

The Bellerophontacea (Figs. 1, 2), remarkable among univalves for their isostrophic, planispirally coiled shells, endured for some 300 million years in the Paleozoic, becoming extinct early in the Triassic. No other group of fossil mollusks has been as subject to as many differing interpretations as have the Bellerophontacea.

To both de Koninck (1843) and Meek (1866), the bellerophontacean slit suggested a relationship to the living families with slits or holes in the shell: the Pleurotomariidae, Haliotidae and Fissurellidae. For over one hundred years this has been the traditional interpretation, accepted by many paleontologists and neontologists. In this century many authors, including Yonge (1947), Knight (1952), Knight *et al.* (1960), Morton & Yonge (1964), Batten *et al.* (1967), Rollins & Batten (1968), and Linsley (1978a), have regarded the bellerophontaceans as the most primitive gastropods, ancestral to all living slit-bearing gastropods.

Another school, basing their evidence on the multiple pairs of muscle scars in some bellerophontiform genera, considers all such forms to have been nontorted and therefore not gastropods but monoplacophorans. This view originated with Wenz (1940) and has been supported by Termier & Termier (1968), Runnegar & Pojeta (1974), Pojeta & Runnegar (1976), Runnegar & Jell (1976), Lever (1979), Salvini-Plawen (1980, 1981), and Runnegar (1981).

A less radical view has been upheld by other paleontologists, who have accepted some bellerophontiform mollusks as nontorted—the "cyclomyan monoplacophorans," but maintain that the Bellerophontacea proper are still to be considered torted. Authors who have argued this interpretation are Knight (1947, 1952), Horný (1963, 1965), Yochelson (1967. 1978, 1979), Rollins (1967, 1969), Rollins & Batten (1968), Starobogatov (1970), Peel (1972, 1974, 1976, 1980), Berg-Madsen & Peel (1978), and Linsley (1978a, 1978b). Varying concepts of the separation between the torted and nontorted members have been discussed by these authors. Moreover, Yochelson has maintained that the Bellerophontacea need not be considered the most primitive gastropods.

Most recently, Harper & Rollins (1982) have argued that muscle scar patterns are not reliable for phylogenetic assessment and have interpreted the cyclomyan group, as well as the bellerophontaceans, as gastropods. The controversy surely continues!

The possibility that fissurellids were derived from bellerophontaceans has great intrinsic interest. It also has bearing on this heated controversy: were the bellerophontaceans torted and therefore gastropods, or, were they nontorted and not gastropods at all? If it can be shown that the fissurellids are derivatives of the bellerophontaceans, then the bellerophontaceans were gastropods.

Background References on Fissurellids and Bellerophontaceans

The genera referred to here are, for the most part, diagnosed and illustrated in the archaeogastropod volume of the *Treatise on Invertebrate Paleontology* (Knight *et al.*, 1960), in which the Paleozoic groups were treated by J. B. Knight, R. L. Batten, and E. L. Yochelson, those of the Mesozoic by L. R. Cox, and those of the Cenozoic by A. M. Keen and R. Robertson. Knight's (1941) *Paleozoic Gastropod Genotypes* provides photographic illustrations useful for comparison with the drawings of shells in the *Treatise*. Because authors, dates, and type-species of genera are readily available in these works, this information is not repeated here.

The classification of Fissurellidae followed here is that of Thiele (1929), in which two subfamilies, the Emarginulinae and the Fissurellinae, are distinguished on the basis of major radular differences. I will further discuss the classification of Fissurellidae in other pa-

pers now in preparation and will suggest that a number of other taxa proposed as subfamilies be recognized at the tribe level within the Emarginulinae.

An overall classification of the Bellerophontacea was provided by Knight *et al.* (1960). Other useful overviews are Lindström's (1884) beautifully illustrated treatment of Silurian gastropods, Horný's (1963) treatment of early Paleozoic bellerophontaceans, and Yochelson's (1960) review of the Permian bellerophontaceans from the southwestern

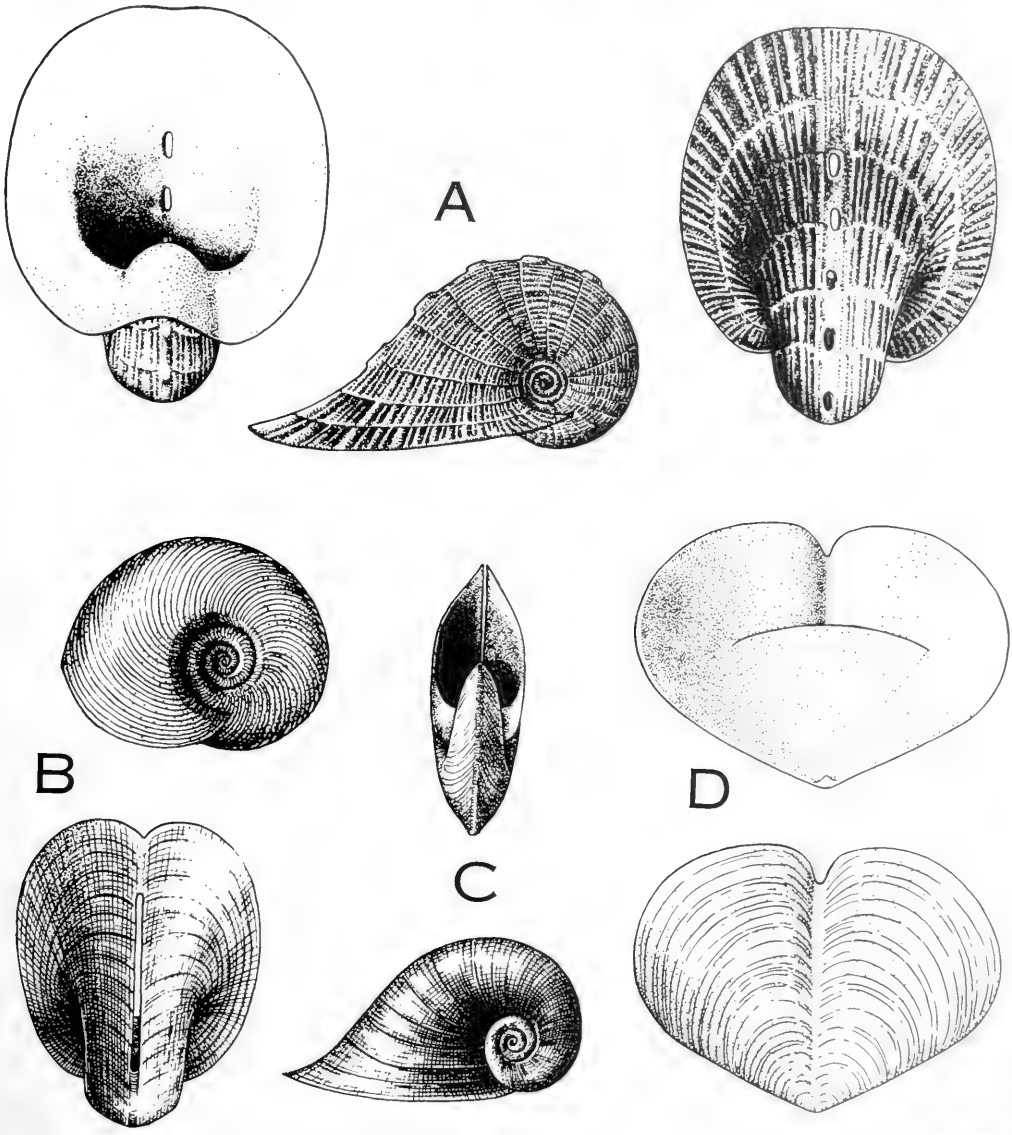

FIG. 1. Bellerophontacean genera typical of the Early Paleozoic. A) *Tremanotus alpheus* Hall, Middle Silurian, 3 views, maximum dimension 77 mm; slit comprised of a row of open tremata, as in *Haliotis*. B) *Salpingostoma boulli* (Whitfield), Middle Ordovician, 2 views, maximum dimension 61 mm; slit closed at margin, as in the fissurellid genus *Rimula*. C) *Tropidodiscus curvilineatus* (Conrad), Lower Devonian, 2 views, maximum dimension 28 mm; slit extremely deep. D) *Pterotheca transversa* (Portlock), Middle Ordovician, 2 views, maximum dimension 44 mm; one of the "crepiduliform" genera with an internal shelf and a terminal apex, resembling the fissurellid genus *Zeidora*. All after Knight *et al.* (1960).

United States. Papers by Peel (1972, 1974, 1975, 1976) are particularly relevant to a functional interpretation of bellerophontaceans. Shells of some representative bellerophontacean genera are illustrated in Figs. 1 and 2.

Fossil Record of the Fissurellidae

Two bellerophontacean genera ranged into the Mesozoic: *Bellerophon* (Knight *et al.*, 1960: 182), which persisted through the Lower Triassic, and *Retispira*, also in the Lower

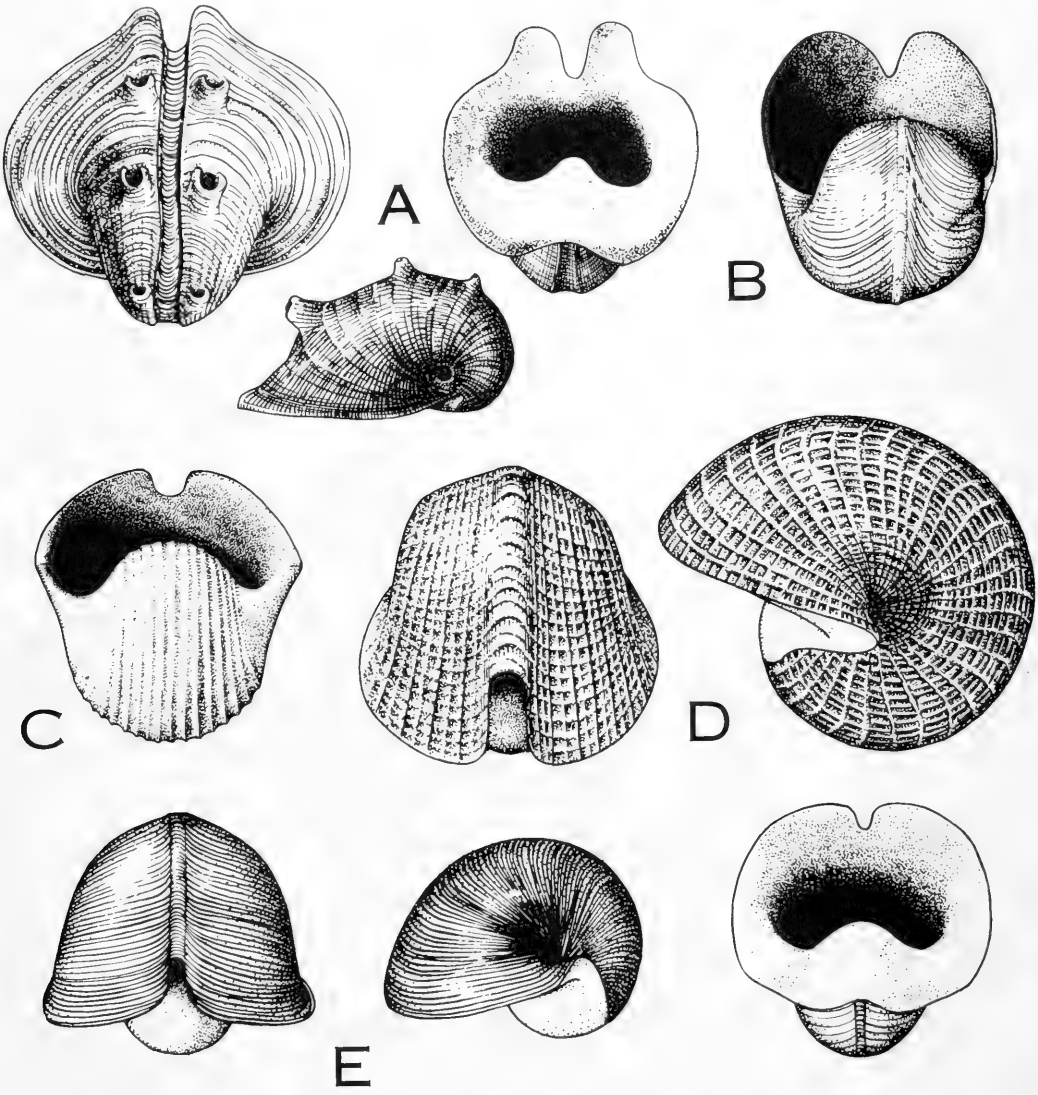

FIG. 2. Bellerophontacean genera typical of the Late Paleozoic. A) *Knightites multicornutus* Moore, Upper Pennsylvanian, 3 views, maximum dimension 39 mm; the protrusions on either side of the selenizone are believed to represent abandoned inhalant canals. B) *Ptychosphaera constricta* Perner, Upper Silurian, maximum dimension 19 mm; one of the genera slightly asymmetrical at later growth stages. C) *Euphemites urii* (Fleming), Lower Carboniferous, maximum dimension 14 mm; a genus likely to have had an internal shell, judging from the breadth and shallow depth of the slit; inductural deposits cover most of the shell. D) *Retispira bellireticulata* Knight, Middle Permian, 2 views, maximum dimension 11 mm; a sculptured genus likely to have had an internal shell. E) *Bellerophon vasulites* Montfort, Middle Devonian, 3 views, maximum dimension 21 mm. All after Knight *et al.* (1960).

Triassic (Yochelson, personal communication). The oldest fissurellid genus—*Emarginula*—is dated by Knight *et al.* (1960) from the Middle Triassic. Thus both groups are scarce in the Triassic.

The Jurassic marks the onset of fissurellid radiation. Illustrations of the fissurellid assemblage described by Huddleston (1887–1896) from the British "Inferior Oolite," of the Bajocian Stage, early Middle Jurassic, are reproduced here (Fig. 3). Genera represented are: *Emarginula,* a genus common in the Recent fauna, *Rimulopsis*, which resembles the living *Rimula* except that the foramen is placed on a broad anterior rib, and *Puncturellopsis*, in which the foramen is somewhat closer to the apex than in *Rimula*. Other fissurellid genera that appeared in the Jurassic were: *Balinula, Emersonia, Koniakaua, Loxotoma, Pseudofissurella,* and *Tauschia*. Of these genera only *Emarginula* now survives, but none of these taxa is very different from living forms.

As noted by Batten (1975: 26), and as is apparent in the Jurassic fissurellids in Fig. 3, shells with high profiles appeared first. By the Eocene, all the modern shell forms, other than those in the subfamily Fissurellinae, were represented in the Paris Basin.

Hypothesis for the Origin of the Fissurellidae

My hypothesis is that the Fissurellidae are limpet descendants of the Bellerophontacea, just as the Haliotidae are limpet derivatives of the Pleurotomariacea. The Fissurellidae differ from the Bellerophontacea primarily in lacking coiling and whorl overlap. Such other features of bellerophontacean organization as shell structure and the major features of internal anatomy are shared with the presumed ancestral group.

Changes in anatomy would have involved the gill structure. In fissurellids and haliotids the ctenidia have short afferent membranes; in the pleurotomariids the mantle cavity is deep, extending even deeper than the bases of the ctenidia. Afferent support to the ctenidia is lacking in pleurotomariids; a deep mantle

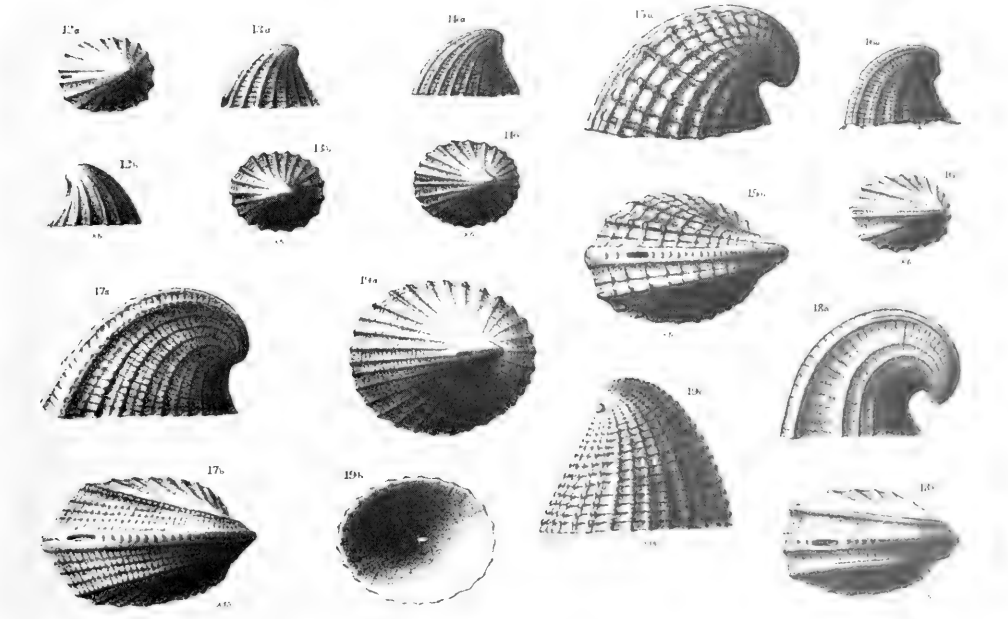

FIG. 3. Early radiation of the Fissurellidae. Copy of part of pl. 41 from Huddleston (1887–1896), showing the fissurellids of the British "Inferior Oolite," of the Bajocian Stage, early Middle Jurassic. Included are species of *Emarginula* (upper left, ×4), a genus with a marginal slit that is well represented in the Recent fauna; the extinct *Puncturellopsis* (lower left center, ×10), a *Rimula*-like genus with the foramen close to the apex; and three species of the extinct *Rimulopsis* (upper right and lower right, ×4; lower left, ×10), in which the foramen is midway on a broad anterior rib.

cavity requires freedom of access to the entire mantle cavity that obtains when ctenidia are attached only ventrally. The gills are close to the columella and therefore all the related pleurotomariid musculature is lateral or ventral to the gills. In haliotids and fissurellids, the mantle cavity is necessarily short and there is no shell surface for support ventral to the gill; dorsal support for the gill, provided by the addition of an afferent membrane, is essential.

It is also probable that bellerophontacean ctenidia lacked afferent membranes. Their coiled shell allowed for a deep mantle cavity in which freedom of access would have required ventral ctenidial attachment only. Ventrally attached bipectinate ctenidia in a bellerophontacean would have been close to the columellar muscles; deployment of these ctenidia therefore would be easily controlled. Thus, the afferent membrane would be a new development in the derivation of fissurellids.[1]

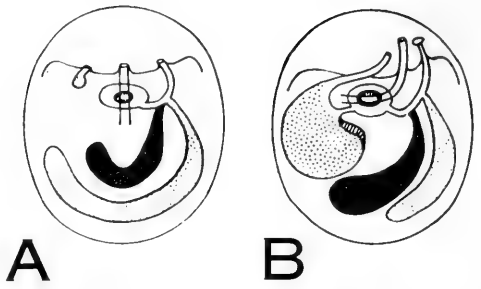

FIG. 4. Diagrammatic representation of the interrelationships of rectum, gonad, pericardial cavity, kidneys and mantle cavity in: A) the fissurellid *Diodora*, and B) the Trochacea (as well as the Pleurotomariidae, Haliotidae, and Scissurellidae). The rectum penetrates the pericardial cavity and ventricle in both. The left kidney is shown opening on the left side, nearly vestigial in *Diodora*, but in the Trochacea it is a large papillary sac with a thickened area of nephridial gland on the side toward the pericardial cavity. In the Trochacea a reno-pericardial duct connects the left kidney and pericardium. In both *Diodora* and the Trochacea the gonad (dark shaded) is connected to the same duct as the right kidney, with openings to the pericardial and mantle cavities. After Fretter & Graham (1962).

EVIDENCE FOR DERIVATION OF FISSURELLIDS FROM BELLEROPHONTACEANS

Unique Aspects of Fissurellid Anatomy

Except for the fissurellids, rhipidoglossate families of archaeogastropods have a characteristic left kidney, a prominent papillary sac (Fig. 4B). This has been described in the Pleurotomariidae (Woodward, 1901; Bouvier & Fischer, 1902; Fretter, 1964, 1966), the Scissurellidae (Bourne, 1908), the Haliotidae (Crofts, 1929, 1937), and the Trochacea (Randles, 1905; Frank, 1914; Risbec, 1939; Fretter & Graham, 1962; Graham, 1965). The papillary sac has a thickened region—the nephridial gland—in its wall on the side adjacent to the pericardial cavity. Renopericardial connections are known in the Trochacea, the Haliotidae, and presumably in the Pleurotomariidae.

The Pleurotomariidae, Haliotidae, and Trochidae have many other features in common, including a nacreous shell interior, large, convoluted hypobranchial glands, and the spiral caecum, an appendage to the stomach. The Trochacea share so many features with the Pleurotomariidae that their derivation from that group is readily perceived (Fretter, 1964, 1966). The Haliotacea, in turn, are the limpetlike derivatives of the Pleurotomariacea.[2]

In contrast, all authors (Boutan, 1885; Illingworth, 1902; Tobler, 1902; Ziegenhorn & Thiem, 1926; Odhner, 1932; Fretter & Graham, 1962) have noted that the reduced left kidney of fissurellids (Fig. 4A) is unique among archaeogastropods. As summmarized by Fretter & Graham (1962: 486): "the left

[1]Although Yonge (1947) considered the presence of an afferent membrane to be primitive in aspidobranch gastropods, the lack of this membrane in the oldest group, the pleurotomariaceans, provides good reason to regard lack of an afferent membrane as primitive.

[2]The Haliotidae were elevated to the rank of superfamily by Golikov & Starobogatov (1975), a decision accepted here. The mantle cavity in the Pleurotomariidae extends deeper than the bases of the ctenidia; in the Haliotidae the mantle cavity is shorter and the ctenidia have afferent membranes. Radular and epipodial characters are also vastly different. The anterior pedal mucous gland, so prominent in pleurotomariids, scissurellids, and trochids, is absent in the Haliotidae. Differences between the Pleurotomariidae and Haliotidae are greater than those among constituent families of either the Trochacea or Patellacea; consequently, the separation of Pleurotomariidae and Haliotidae at the superfamily level is justified and is consistent with the superfamilial distinction between the Bellerophontacea and Fissurellacea proposed here, as well as that between the hypothesized condition in the extinct Euomphalacea and the living Neomphalacea (McLean, 1981).

kidney is minute, if not functionless. . . The right is responsible for all the excretory activity and also acts as a conduit for the genital products." Illingworth (1902: 458) described the left kidney of *Megathura crenulata* (Sowerby), as "a brownish spot in the anterior wall of the pericardial cavity."

Further reductions of other archaeogastropod structures in fissurellids include hypobranchial glands that are reduced to gland cells within the mantle skirt and on the ctenidial axes, and the spiral caecum of the stomach reduced to a vestige. Owen (1958) found other modifications in the stomach that suggested secondary simplicity, compared to the condition in the Trochacea.

Other unique anatomical states in the Fissurellidae include: the capacity of the middle fold of the mantle to envelop the shell (discussed below), a diverticulum extending ventrally between the two halves of the buccal mass (Fretter & Graham, 1962: fig. 96), and "the separation of the intestinal groove from the main part of the intestine" (Fretter & Graham, 1962: 615).

Fissurellid anatomy so differs from that of the three other living dibranchiate families—the Pleurotomariidae, Haliotidae, and Scissurellidae—that it suggests no close relationship to these families. The Fissurellidae can be interpreted as sharing the basic anatomy of a coiled predecessor in which the anatomy also differed from that of any living family. The extinct pleurotomariaceans are sufficiently similar in shell morphology to those now living to suggest that their anatomies were also like that of living forms. Because shell form in the Bellerophontacea differs from that of the Pleurotomariacea, the Bellerophontacea are prime candidates as ancestors of the fissurellids.

If the condition of the left kidney of the fissurellids was shared with the bellerophontaceans, the latter would have been outside the main line of gastropod evolution. The vestigial condition of the fissurellid left kidney means that most of the excretory function is provided by the right kidney. Modification of the right kidney system for reproductive specialization would be less likely in a lineage in which it must also assume the entire excretory burden. In prosobranchs, the change from the archaeogastropod to mesogastropod grade includes the modification of the right kidney to form a more advanced reproductive system with glandular gonoducts (Fretter & Graham, 1962; Fretter, Graham & McLean, 1981). However, if the bellerophontacean kidney had the same limitation as has the fissurellid kidney, experimentation with the limpet form was still possible.

Shell Structure in Fissurellids and Bellerophontaceans

Shell structure in the fissurellids is unlike that of the pleurotomariaceans or haliotids in lacking a nacreous inner layer. According to Bøggild (1930: 300), fissurellid shell structure may be characterized by having "a crossed lamellar layer with concentrical lamellae and in most instances that layer forms the whole shell." Bøggild's analysis was confirmed by MacClintock (1963). The crossed-lamellar shell structure of *Fissurella latimarginata* Sowerby is illustrated here (Fig. 5A).

The phylogenetic significance of shell structure is difficult to evaluate because the shells of most Paleozoic fossils are altered or replaced, destroying all traces of structure. Nothing is known about shell structure in either the cyclomyan group or the oldest bellerophontiform mollusks. Nevertheless, some knowledge of shell structure in bellerophontaceans has been contributed by MacClintock (1967), Rollins (1967), and Batten (1982). Batten has recently found considerable diversity in the group, though MacClintock and Rollins found only crossed lamellar aragonite or complex crossed lamellar aragonite in late Paleozoic bellerophontaceans. MacClintock (1967) concluded that "the bellerophontins are more closely related to the fissurelloids than they are to the pleurotomarioids."

MacClintock's conclusion about a possible relationship between fissurellids and bellerophonts has not been mentioned by subsequent authors, including Golikov & Starobogatov (1975).

Derivation of the fissurellids from a pleurotomariacean ancestor would require a basic change in shell structure. This would not be required for derivation of fissurellids from bellerophontaceans. Therefore, the parsimonious route is derivation of fissurellids from a late Paleozoic bellerophontacean ancestor.

Coiling and Symmetry in Fissurellids and Bellerophontaceans

Most authors have claimed that ontogeny in fissurellids includes a trochospirally coiled

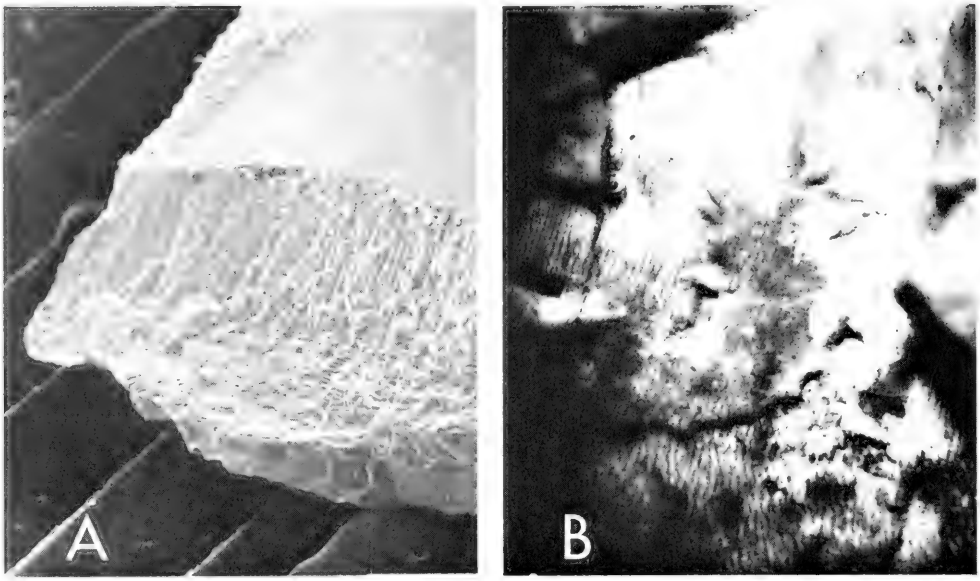

FIG. 5. Crossed-lamellar aragonite shell structure in fissurellids and bellerophontaceans. A) *Fissurella latimarginata* Sowerby, fragment of a shell from Iquique, Chile, SEM micrograph showing the smooth internal surface and a fractured edge, the lamellae clearly visible; ×70, courtesy H. Lowenstam. B) The bellerophontacean *Euphemites vittatus* (McChesney), a fragment showing the "first-order lamellae trending nearly straight across selenizone midway through inner shell layer"; ×30, after MacClintock (1967).

phase. Yonge (1947: 458) stated: "The shell is secondarily symmetrical, although spiral twisting is apparent during development." In support of this statement, Yonge copied a figure from Boutan (1885), identified by that author as a fissurellid developmental stage. However, I identify the figure as that of *Scissurella costata* Orbigny (see Fig. 6 and caption for further details). It seems that this misidentified figure has unduly influenced the current understanding of fissurellid development. It was reproduced by Yonge (1947, fig. 8a); Crofts (1955: 747) referred to it as proof that larval fissurellids have a "well developed helicoid spire." Batten (1975, fig. 25), following these authors, copied it, and cited it as evidence that fissurellid ontogeny may include a coiled stage of several whorls, resembling the coiling of a mature scissurellid.[3]

Bandel (1982) also dismisses the so-called "*Scissurella*-stage" in fissurellid ontogeny, but continues to maintain that there is a "trochospirally coiled secondary shell." There is clearly an indication of postprotoconch asymmetry in many fissurellids, as can be seen in many of Bandel's illustrations as well as such previously published illustrations as those of Boutan (1885: pl. 43, 44), Batten (1975: figs. 26–28), Fretter & Graham (1976: figs. 10, 12), and Boggs (1978). The asymmetrically coiled part of the postprotoconch whorl may amount to one-half whorl, but, in my opinion, this is not to be equated with the regular orthostrophic coiling of *Scissurella* or other trochospirally coiled groups.

I have examined the early whorls of a number of fissurellid species and find that postprotoconch form varies from nearly symmetrical to a more frequent condition in which the apex is offset toward the right (in dorsal view). Nearly symmetrical shells at all growth stages are characteristic of some species of *Emarginula* (Figs. 7A, B). In *Diodora* there is a slight to moderate projection toward the right (Figs. 7C, D). Many species of *Fissurella* are nearly symmetrical (Figs. 7E, F).

Bellerophontaceans are unique among gastropods for their nearly perfect symmetry

[3]Batten (1975) theorized that Scissurellidae were neotenously derived from the Fissurellidae, basing his conclusion in part upon the supposed "*Scissurella*-stage" of fissurellids. Bandel (1982) refuted a close relationship between the Scissurellidae and Fissurellidae on other grounds. The fact that the left kidney of the Scissurellidae is a papillary sac (Fretter & Graham, 1962, 1976) provides reason enough to dissociate the two families.

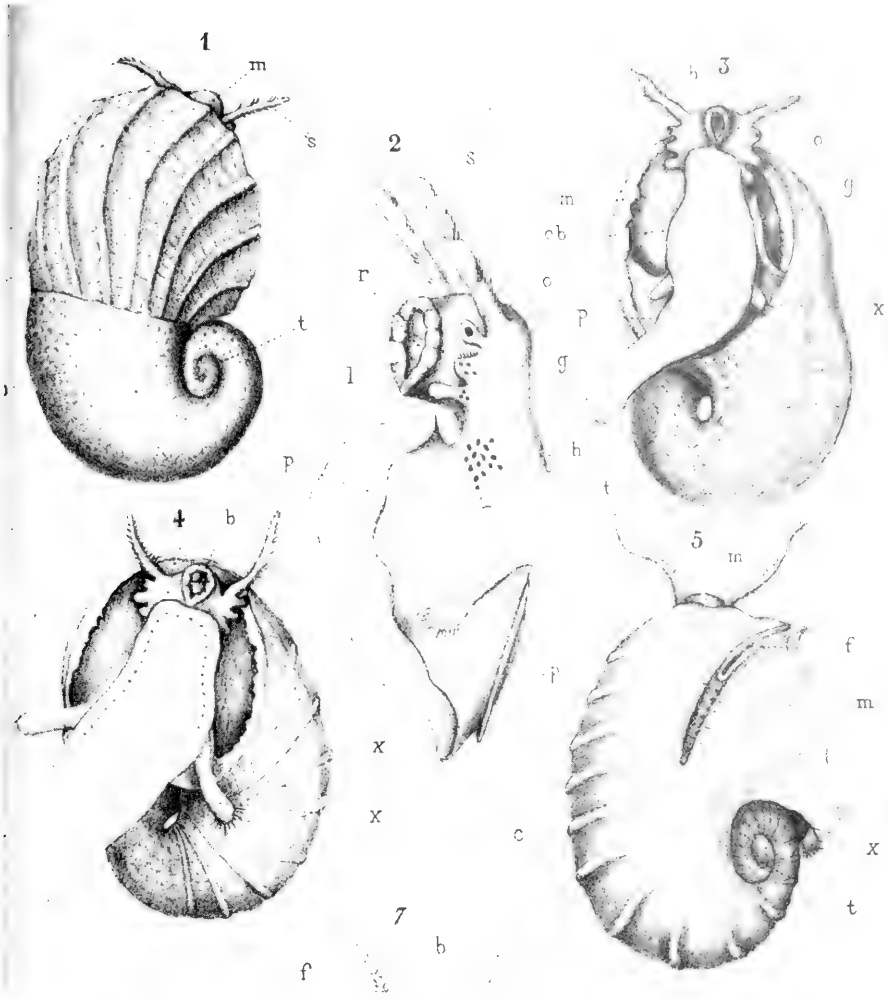

FIG. 6. Copy of part of Boutan's (1885) pl. 42, showing two different juvenile shells purported to represent the "Développement de la Fissurelle." The fissurellid species studied by Boutan is now known as *Diodora apertura* (Montagu). Boutan's figs. 1 and 3 show the early sculpture of *D. apertura*, but I identify the shell in figs. 4 and 5 as *Scissurella costata* Orbigny, a species that occurs at Banyuls-sur-Mer, the Mediterranean locality at which Boutan worked. Vayssière (1894) studied *Scissurella costata* at the same locality. Although Boutan gave no magnification, the axial ribs on his figure may be matched with those in a correctly identified figure of *S. costata* in Batten (1975: fig. 12). In both the Boutan figure (copied by Batten, fig. 25) and Batten's figure 12, the axial sculpture is present for exactly 1⅕ whorl preceding the first appearance of the slit. Boutan made no mention of the discrepancy among the shells figured on pl. 42, either in his detailed caption or in the text. Perhaps his artist made the error, which he failed to notice. Although I identify the shell in Boutan's figs. 4 and 5 as that of a *Scissurella*, the animal depicted in these figures is not *Scissurella*, but is in agreement with his other illustrations of early stages in *Diodora apertura*.

at all growth stages. However, some genera, such as *Ptychosphaera* (Fig. 2B), are asymmetrical in the final whorl.

Few authors have discussed bellerophontacean protoconchs. Dzik (1978: 295, fig. 4D) illustrated—without giving magnification—a supposed Ordovician bellerophontacean protoconch (and first postprotoconch whorl) identified as "*Modestospira*? sp." Peel (1974) described ontogeny in the Silurian bellerophontacean genera *Plectonotus* and *Tritonophon*, based upon "transverse

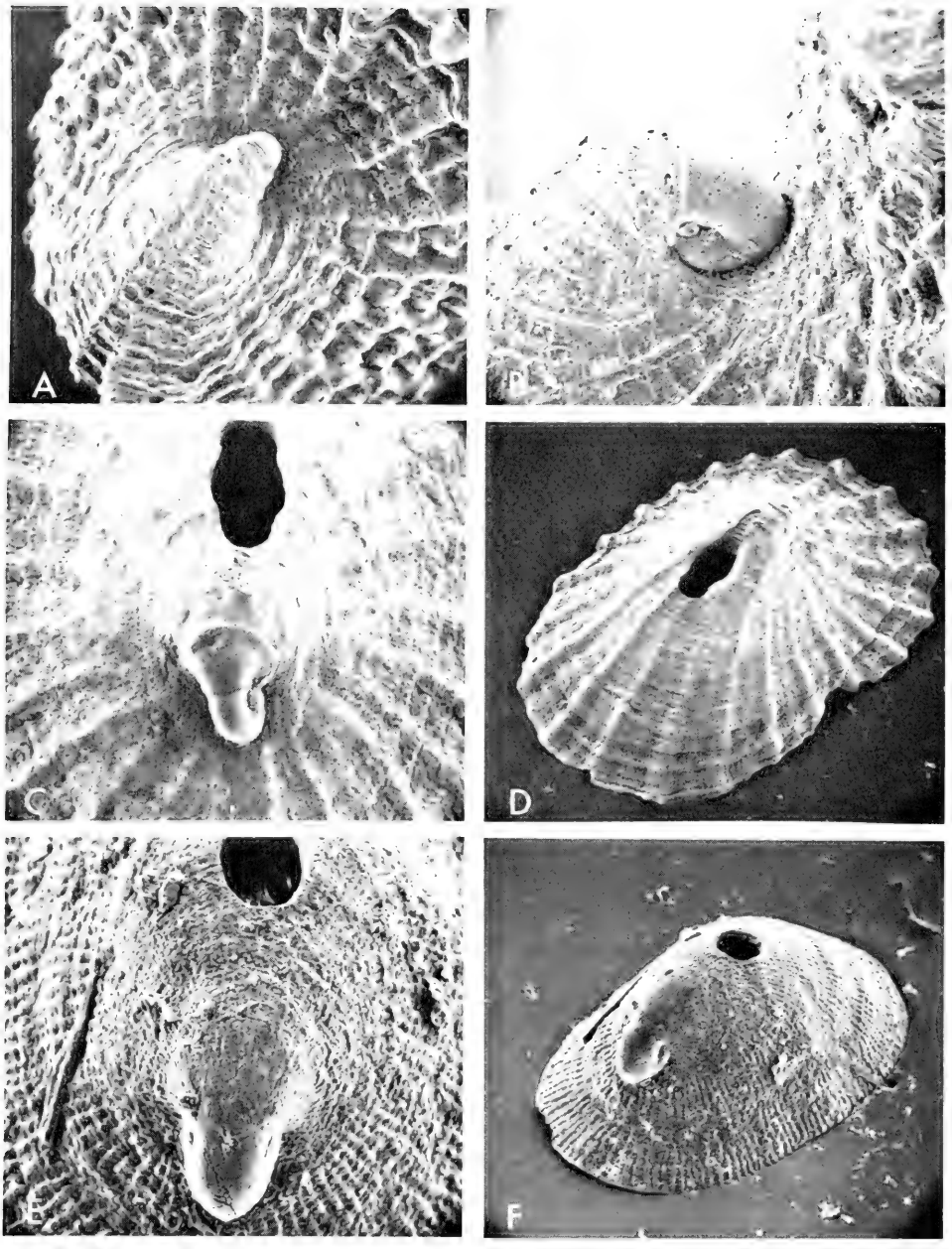

FIG. 7. SEM micrographs showing protoconchs, selenizones, or foramina of fissurellid species (SEM stubs in LACM collection). A & B: *Emarginula superba* Hedley & Petterd, 183 m off Cape Pillar, Tasmania, shell length 3.2 mm. A) Apical view, showing minimal asymmetry and origin of the selenizone less than one protoconch diameter from the protoconch lip, B) lateral view of protoconch from left side. C & D: *Diodora inaequalis* (Sowerby), Guaymas, Sonora, Mexico, shell length 1.9 mm. C) Dorsal view, showing the first appearance of the selenizone at two protoconch diameters away from protoconch lip, D) oblique anterior view of entire shell. E & F: *Fissurella rubropicta* Pilsbry, Cabo San Lucas, Baja California, Mexico, shell length 0.5 mm. E) Dorsal view, showing bilateral symmetry of protoconch and early postprotoconch shell, F) oblique lateral view of entire shell, showing the early foramen two protoconch diameters from the protoconch lip; selenizone lacking at all stages.

cross-sections of specimens, cut so as to contain the axis of coiling and to be perpendicular to the plane of symmetry." Cross-sections of early whorls were "subcircular," with no indication of asymmetry. More needs to be said about bellerophontacean protoconchs; meanwhile, there is no indication that they are atypical of gastropods.

Even if bellerophontacean protoconchs are completely symmetrical, this need not preclude them from being torted gastropods, for there are coiling variations in gastropods that differ from the usual condition for dextral forms. In some cases the anatomy can be dextral, but the shell is sinistral—this is known as hyperstrophic coiling (Cox, 1960: 110; McLean, 1981: 315). Hyperstrophy in living prosobranchs is known in the ampullariid genus *Lanistes* (see Cox, 1960: fig. 67) and in the planktotrophic veliger stages of the Architectonicidae (Robertson, 1964). If torsion in anatomically dextral forms can produce sinistral shells, then the intermediate planispiral condition should also be possible following torsion.

The postprotoconch asymmetry of fissurellids does not agree with the nearly perfect symmetry of most bellerophontaceans. My explanation of bellerophont symmetry is that—for reasons unknown—the inherent asymmetry of torsion was masked. However, in the limpet derivative, in which extensive coiling in the adult does not take place, the masking effect is removed in conjunction with the suppression of coiling. The adult symmetry of fissurellids is shared with the ancestral group.

Whorl Overlap in Fissurellid Ontogeny and in Mature Bellerophontaceans

Boutan (1885) emphasized that evolution in the Fissurellidae is revealed by the shell ontogeny of advanced genera, in which the foramen first appears as a marginal slit—the "*Emarginula*-stage"—followed by the "*Rimula*-stage," in which the slit closes at the margin, placing the foramen midway on the anterior slope, and progressing to the "*Diodora*-stage," in which the foramen obliterates the shell apex. I carry this analysis one step further and note that the early stage of the fissurellid resembles that of a mature bellerophontacean: the early postprotoconch shell bulges along the columellar side of the aperture in the first postprotoconch whorl of *Diodora* (Fig. 8). In a mature bellerophontacean the previous whorl obstructs the aperture—the "whorl overlap," discussed by Linsley (1978b: 199)—which characterizes bellerophontaceans at all growth stages.

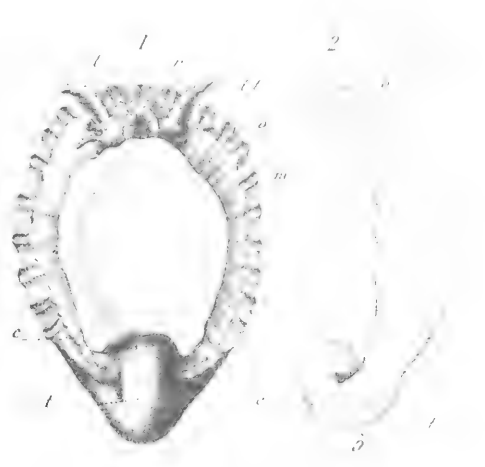

FIG. 8. Whorl overlap in fissurellid ontogeny, resulting from delayed growth along the columella in the fissurellid postprotoconch. Two views of *Diodora apertura* (Montagu), "rimuliform larva" in ventral view. After Boutan (1885), no original magnification given.

The whorl overlap in fissurellid ontogeny results from retardation of growth along the columellar lip, as is apparent in illustrations of Boutan (1885) and Boggs (1978). In his description of development in the Hawaiian species *Diodora granifera* (Pease), Boggs (1978) reported that only at the age of three weeks does the shell begin to grow along the columellar margin, the same stage in development at which the exhalant notch is enclosed to form a foramen. This is a different course of shell development from that taken by trochids, in which growth along the columellar lip begins immediately, as may be seen in views of larval trochid shells illustrated by Bandel (1975: pl. 1).

In my interpretation of fissurellid ontogeny, all but the final whorl of the bellerophontacean is reduced to the first postprotoconch whorl of the fissurellid. I therefore see a bellerophont stage in the ontogeny of the Fissurellidae.

Muscles and Muscle Scars in Fissurellids and Bellerophontaceans

Fissurellid muscle scars (Figs. 9B, C, D) differ from the generalized horseshoe-shaped muscle scars of most limpets in that both

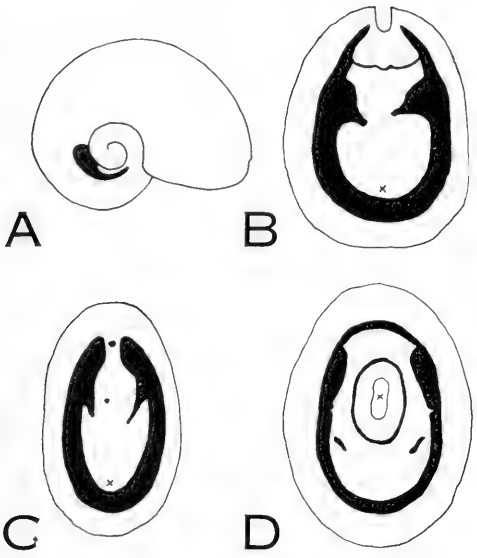

FIG. 9. Muscle scars in a bellerophontacean (A, after Peel, 1980) and fissurellids (B–D, after MacClintock, 1963; x marking the position of the apex). A) Diagrammatic representation of muscle scar in *Bellerophon*, lateral view. B) Muscle scar of *Emarginula candida* A. Adams, showing the inwardly directed hook-shaped process; shell length 10 mm. C) Muscle scar of *Tugali parmophoidea* (Quoy & Gaimard), showing the hook-shaped process; shell length 15 mm. D) Muscle scar of *Fissurella volcano* Reeve, a member of the Fissurellinae, the most advanced fissurellid group, in which the hook-shaped process is lost; shell length 24 mm.

FIG. 10. Lateral view of the "right muscle scar of *Bellerophon* specimen B" of Peel (1972, text fig. 1) aperture opening toward the right, ×6. S1 and S2 represent the markings of the "oblique transdorsal swellings," which migrate anteriorly with growth, and are here considered as possibly homologous with the internally directed "hook-shaped process" of the fissurellid muscle scar. See Peel (1972) for a more detailed description of this muscle scar configuration.

lobes have a posteriorly and inwardly directed hook-shaped appendage (except in the subfamily Fissurellinae), as diagrammed by MacClintock (1963: figs. 21–29). In drawings of fissurellid anatomy (Odhner, 1932; Yonge, 1947: fig. 11; Fretter & Graham, 1962: figs. 254, 257), it is clear that these posteriorly directed processes of the muscle are in close proximity to the ctenidial axes and serve to define the posterior-lateral extent of the mantle cavity. The hooked processes of the muscle may therefore aid in the positioning of the ctenidia.

Paired columellar muscle scars in bellerophontaceans (Figs. 9A, 10) have been documented by Knight (1947) and more recently in a number of additional bellerophontacean genera by Rollins (1967) and Peel (1972, 1974, 1976). Peel (1972: 415) described faintly indicated "oblique transdorsal swellings." Peel's illustration is copied here (Fig. 10). Their significance was not understood: "While the location of the various swellings on the moulds demonstrates some connection with the musculature, the nature and purpose of the structure is quite unknown."

Although Peel did not know the significance of the "oblique trans-dorsal swellings" he observed, I suggest that their homology with the posteriorly directed process of the fissurellid muscle be considered. If these transdorsal markings serve to define the posterior extent of a bellerophontacean mantle cavity, they would necessarily be transitory and weakly impressed in an actively growing bellerophontacean because with growth, the muscle scar would be migrating continually toward the aperture.

In a derivation of fissurellids from bellerophontaceans, the loss of the columella would force an anterior migration of the paired retractor muscles and their posterior union to form the horseshoe-shaped fissurellid muscle. The "oblique-transdorsal element" of the bellerophontacean muscle would be retained as the inwardly directed hook-shaped process of the fissurellid muscle, which defines the posterior extent of the mantle cavity.

Apertural Slits in Fissurellids and Bellerophontaceans

The Fissurellidae show a gamut of possible expressions of the apertural slit: a weakly indented sinus in *Scutus*, a slightly raised

sinus in *Hemitoma*, a clearly delineated selenizone in *Emarginula* (Figs. 7A, 7B) and *Zeidora*, a foramen on the anterior slope in *Rimula*, and an apical perforation in *Diodora* and *Fissurella*. The selenizone is present only in the postprotoconch stage of *Diodora* (Figs. 7C, D), whereas in *Fissurella* (Figs. 7E, F), the foramen appears without leaving a selenizone at any stage.[4]

Bellerophontaceans also display a wide range of possible expressions of the slit. An extremely deep slit was represented in *Tropidodiscus* (Fig. 1C), which ranged from the Lower Ordovician through Devonian. The Ordovician-Silurian *Salpingostoma* (Fig. 1B) had a foramen on the anterior slope much like the fissurellid *Rimula*, and the Silurian *Tremanotus* (Fig. 1A) had a row of open tremata, a condition parallel to that in the Haliotidae. The Ordovician *Pterotheca* (Fig. 1D) had a raised U-shaped sinus and the shell interior had a shelf, a feature convergent with the interior shelf of the Recent fissurellid *Zeidora*.

The presence of a slit and a slitband or selenizone in a living gastropod is an indication that the animal is a gastropod having paired ctenidia, with the single exception of the mesogastropod family Siliquariidae (see Gould, 1966).

Because slits and slitbands are characteristic of virtually all dibranchiate gastropods, the argument that the fissurellids have a slit and a slitband suggests that they could have been derived either from pleurotomariaceans or bellerophontaceans.

Envelopment of Shell Margin in Fissurellids and Bellerophontaceans

Fissurellids have a capacity unique in the Gastropoda—the mantle can envelop the shell without causing a corresponding obliteration of exterior sculpture. In other families with enveloped shells, the mantle produces a glossy external surface, as in the Cypraeidae and Olividae. As detailed by Stasek & McWilliams (1973), the fissurellid mantle margin has three folds. The outer fold secretes the growing edge of the shell, the inner fold extends down to envelop the foot, and the middle fold is capable of partially enveloping the shell, as in *Emarginella* (Fig. 11), or completely enveloping it, as in *Megathura* (Fig. 12). Such fissurellids as *Diodora* usually envelop only the shell edge with the middle fold, but can fully envelop the shell in defense against sea stars (Margolin, 1964).

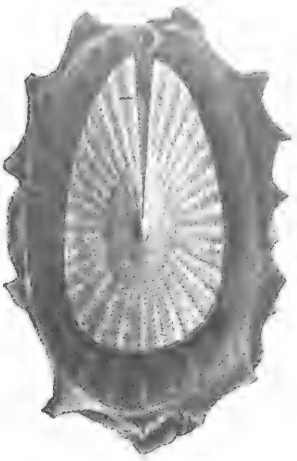

FIG. 11. Shell envelopment in the fissurellid *Emarginella clypeus* (A. Adams), dorsal view, anterior at top. The shell is half-way enveloped by the middle fold of the mantle and the mantle margin is split anteriorly corresponding to the depth of the slit. Overall length about 2 cm. After Schepman (1908).

FIG. 12. Shell envelopment in the fissurellid *Megathura crenulata* (Sowerby), the shell completely enveloped by the middle fold of the mantle, except for a narrow exposed portion at the foramen; the inner fold of the mantle lifted to show the paired ctenidia, the head with cephalic tentacles, and the foot. Overall length about 12 cm. After Stasek & McWilliams (1973).

[4]*Fissurella* lacks a selenizone at all stages (see Bandel, 1982, pl. 12, fig. 8, pl. 12, 10; Batten, 1975, fig. 26; Fig. 8F here), providing a taxonomic character significant at the subfamily level in separating the Fissurellinae from other fissurellids. Bandel's figure (1982: pl. 10, fig. 6), identified as an early shell of *Fissurella nimbosa*, is evidently a misidentification of a species of *Diodora*, for it shows a selenizone preceding the foramen.

Some fissurellid genera have a shell that is greatly reduced compared to the size of the body. *Scutus* has the shell edge permanently enveloped by the mantle, which can expand to fully cover the shell. *Laevinesta atlantica* (Pérez Farfante) has a completely internal shell; the body is three times longer than the shell (Pilsbry & McGinty, 1952, and personal observation).[5] The shell of *Laevinesta* is centrally positioned and the gills extend well forward of the shell. In *Scutus, Emarginella,* and *Laevinesta* the mantle margin is split anteriorly to a depth corresponding to the position of the apertural slit, where it forms an excurrent siphon. The selenizone is much broader in both *Emarginella* and *Laevinesta* than in *Emarginula*, a genus in which the shell and body size are equivalent. In the latter genus the entire apertural margin is contained in the same plane.

For the genera with an apical foramen, shell envelopment occurs in *Lucapina* and *Megathura* and is especially pronounced in *Pupillaea* and *Fissurellidea*. In all these genera the body is much larger than the shell, the foramen is relatively large, the mantle cavity and ctenidia project in front of the shell, and the gills are covered only by the greatly thickened mantle fold.

The following features of fissurellid anatomy can usually be deduced from shells: saddle-shaped shells with raised ends correlate with greatly reduced or internal shells; broad slit bands or large foramina also correlate with internal shells; shells with the entire apertural margin in the same plane correlate with minimal shell envelopment by the mantle.

Some bellerophontaceans have recently been considered to have had internal shells, particularly those with radial (rather than oblique or tangential) apertures (Linsley, 1977, 1978b). The inductural (callus) deposits of *Euphemites* were also stressed by Linsley (1978b) in his argument that *Euphemites* had an internal shell. He rendered it with an animal about four times larger than the fully enveloped shell (Fig. 13).

FIG. 13. Linsley's (1978b) reconstruction of the bellerophontacean *Euphemites* with an internal shell. Overall length about 6 cm.

An explanation for the exceptionally broad slit bands of some bellerophontaceans (Figs. 2C, D) is that bodies were much larger than the internal shells, as is those fissurellids with broad slit bands or large foramina. As in fissurellids with apertures in the same plane, bellerophontaceans with tangential apertures are likely to have had the least amount of shell envelopment by the mantle, whereas those in which the aperture does not make a plane are almost certainly those in which the shell margin was fully enveloped, if not completely internal.

There is little sculptural difference between fissurellids and many bellerophontaceans; both may have similar cancellate sculpture. An internal shell in a bellerophontacean with intricate reticulate sculpture like that of *Retispira* can be attributed to a mantle edge like that of fissurellids. I reconstruct *Retispira*, with an internal shell (Figure 14). The radial aperture of *Retispira* also suggests that it had an internal shell. Unlike Linsley's drawing of *Euphemites,* my reconstruction of *Retispira* shows mantle folds like those of fissurellids.

Shell envelopment is also possible in the Haliotidae and Trochidae, groups of pleurotomariacean affinity. *Haliotis asinina* Linnaeus has an epipodial fold that expands over a smooth shell surface. The trochid *Stomatella* (subfamily Stomatellinae) has a smooth, enveloped shell. Because shell envelopment in genera of pleurotomariacean-trochacean affinity involves a loss of surface sculpture, a derivation of Fissurellidae from that source is unlikely.

CONCLUSIONS

The origin of all limpet groups can be regarded as paedomorphic—retention of an-

[5]The radula of *Laevinesta atlantica*, unlike the anatomical characters, is so unusual that Hickman (1983) doubted its fissurellid affinity. She compared the radula to that of *Clypidina*. However, shell and mantle characters of *Laevinesta* are more like those of *Emarginella* (Fig. 11), in that both have darkly pigmented bodies and rather flat, enveloped shells with broad selenizones. Subsequent to her publication, I have examined a light microscope preparation of the radula of an unidentified species of *Emarginella* (USNM 235814) and have noted that the rachidian and lateral teeth have obtusely pointed overhanging tips on which there are fine denticles, a condition that could be further modified to produce the strongly cusped condition of *Laevinesta*. Although radular differences are still extreme, I maintain that a derivation of the *Laevinesta* radula from that of *Emarginella* is possible.

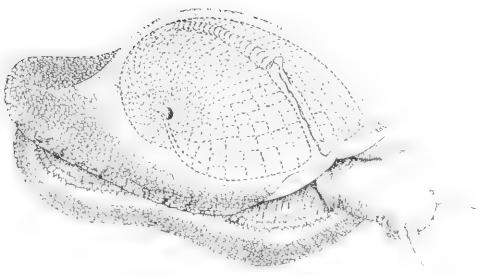

FIG. 14. Reconstruction of the bellerophontacean *Retispira* with an internal shell covered by mantle folds like those of fissurellids. The ctenidia project forward of the shell aperture under cover of the inner fold of the mantle, which would be capable of completely enveloping the head and foot. A cleft in the mantle extends the depth of the apertural slit, marking the position of the exhalant siphon. Epipodial tentacles like those of fissurellids are shown in a row along the side of the foot and neck. The upper surfaces of the foot and mantle are rendered with a textured surface like that of many living fissurellids. Overal length about 2 cm. Drawing by Mary Butler.

cestral juvenile characters by later ontogenetic stages of descendants (see Gould, 1977)—in the sense that limpets are sexually mature postlarval gastropods because they remain uncoiled. If the Fissurellidae are the evolutionary result of the suppression of bellerophontacean coiling, it will be nearly impossible to find transitional forms in the fossil record. A paedomorphic transition acts upon the developmental process and has to be a rapid event. The potential would have existed in any bellerophontacean stock.

In the absence of transitional forms, evidence for a derivation of fissurellids from bellerophontaceans is indirect: 1) Fissurellid anatomy, which differs extensively from that of the pleurotomariids and haliotids, could be a reflection of the condition in the bellerophontaceans. 2) The similarity of fissurellid shell structure to that of the late Paleozoic bellerophontaceans suggests common ancestry. 3) The postprotoconch asymmetry of fissurellids can be explained as an unmasking of the inherent asymmetry of torsion, following the loss of coiling. 4) Delayed onset of growth on the postprotoconch columellar lip of the fissurellid causes whorl overlap at this stage, which resembles the whorl overlap of mature bellerophontaceans, suggesting that fissurellid phylogeny is revealed in its ontogeny. 5) The hook-shaped process of the fissurellid muscle scar may be homologous to the "oblique transdorsal element" of the bellerophontacean muscle scar. 6) The slit in fissurellids and bellerophontaceans is homologous, although it is also homologous with those of the Pleurotomariacea. 7) The unique mantle edge of fissurellids enables the retention of surface sculpture on enveloped shells; a similar capacity in bellerophontaceans would be compatible with their intricately sculptured shells, which in many were probably internal.

Fissurellids are unique in having pores that extend through the shell, particularly in the early stages of the adult shell, as detailed by Bandel (1982). Bandel attaches little phylogenetic significance to these pores; however, if they were to be detected in bellerophontaceans, this would present a powerful argument for the derivation of fissurellids from the bellerophontaceans.

Despite the indirect nature of the evidence, I conclude that it favors a derivation of the Fissurellacea from the Bellerophontacea. I therefore transfer the Fissurellacea from the suborder Pleurotomariina to the suborder Bellerophontina Cox & Knight, 1960. It therefore follows, in agreement with Yochelson (1967, 1978, 1979), that the suborder Bellerophontina is not regarded as comprising primitive gastropods, but is instead an offshoot of the Pleurotomariina in which the internal anatomy differs (as in living fissurellids) from the primitive pleurotomariacean condition.

It is beyond the scope of the present paper to consider the arguments of the "bellerophont controversy," as that has been thoroughly treated by authors mentioned earlier. I find the arguments of paleontologists who regard bellerophontaceans as gastropods the most convincing, noting also that no author supporting the non-gastropod interpretation of bellerophontaceans has provided a reconstruction of the mantle cavity to show how those genera with deep slits would function with the slit in the posterior position.

If the Fissurellidae were derived from late Paleozoic Bellerophontacea, the latter, at least, must have been torted. It also follows that further clues to the form and function of bellerophontaceans can be provided by the living fissurellids.

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LITERATURE CITED

BANDEL, K., 1975, Das Embryonalgehäuse mariner Prosobranchier der Region von Banyuls-sur-mer. *Vie et Milieu*, ser. A, 25: 83–118.

BANDEL, K., 1982, Morphologie und Bildung der frühontogenetischen Gehäuse bei conchiferen Mollusken. *Facies, Universität Erlangen*, 7: 1–198, pl. 1–22.

BATTEN, R. L., 1975, The Scissurellidae—are they neotenously derived fissurellids? *American Museum Novitates*, 2567: 1–29.

BATTEN, R. L., 1982, The origin of gastropod shell structure. *Third North American Paleontological Convention, Proceedings*, 1: 35–38, 1 pl.

BATTEN, R. L., ROLLINS, H. B. & GOULD, S. J., 1967, Comments on "The adaptive significance of gastropod torsion." *Evolution*, 21: 405–406.

BERG-MADSEN, V. & PEEL, J., 1978, Middle Cambrian monoplacophorans from Bornholm and Australia, and the systematic position of the bellerophontiform molluscs. *Lethaia*, 11: 113–125.

BØGGILD, O. B., 1930, The shell structure of the mollusks. *Det Kongelige Danske Videnskabernes Selskabs Skrifter, Niende Raekke, Naturvidenskabelig og Mathematisk Afdeling* 9, *Raekke* 2: 231–326, pl. 1–15.

BOGGS, C. H., 1978, Development of Hawaiian *Diodora*. *Hawaiian Shell News*, 26(10): 3–4.

BOURNE, G. C., 1908, On the anatomy and systematic position of *Incisura (Scissurella) lytteltonensis*. *Quarterly Journal of Microscopical Sciences*, 55: 1–47, pl. 1–5.

BOUTAN, L., 1885, Recherches sur l'anatomie et le dévelopment de la Fissurelle. *Archives de Zoologie Expérimentale et Générale*, ser. 2, tome 3, memoir 4: 1–173, pl. 31–44.

BOUVIER, E. L. & FISCHER, H., 1902, L'organization et les affinités des gastéropodes primitifs d'après l'étude anatomique du *Pleurotomaria beyrichii*. *Journal de Conchyliologie*, 50: 117–272.

COX, L. R., 1960, Gastropoda—General Characteristics of Gastropoda. *In* MOORE, R. C., ed., *Treatise on Invertebrate Paleontology*, Part. I, Mollusca 2: 84–169.

COX, L. R. & KNIGHT, J. B., 1960, Suborders of Archaeogastropoda. *Proceedings of the Malacological Society of London*, 33: 262–264.

CROFTS, D. R., 1929, *Haliotis*. *Proceedings and Transactions of the Liverpool Biological Society*, 43, Appendix II: 1–174, 8 pl.

CROFTS, D., 1937, The development of *Haliotis tuberculata*, with special reference to organogenesis during torsion. *Philosophical Transactions of the Royal Society of London*, ser. B, 228: 219–268.

CROFTS, D., 1955, Muscle morphogenesis in primitive gastropods and its relation to torsion. *Proceedings of the Zoological Society of London*, 125: 711–750.

DZIK, J., 1978, Larval development of hyolithids. *Lethaia*, 11: 293–299.

EALES, N. B., 1950, Secondary symmetry in gastropods. *Proceedings of the Malacological Society of London*, 28: 185–196.

FRANK, E. J., 1914, Beiträge zur Anatomie der Trochiden. *Jenaische Zeitschrift für Naturwissenschaft*, 51: 377–486, pl. 5.

FRETTER, V., 1964, Observations on the anatomy of *Mikadotrochus amabilis* Bayer. *Bulletin of Marine Science*, 14: 172–184.

FRETTER, V., 1966, Observations on the anatomy of *Perotrochus*. *Bulletin of Marine Science*, 16: 603–614.

FRETTER, V. & GRAHAM, A., 1962, *British prosobranch molluscs; their functional anatomy and ecology*. Ray Society, London, xiv + 755 p.

FRETTER, V. & GRAHAM, A., 1976, The prosobranch molluscs of Britain and Denmark. Part 1—Pleurotomariacea, Fissurellacea, and Patellacea. *The Journal of Molluscan Studies*, Supplement 1: 1–37.

FRETTER, V., GRAHAM, A. & McLEAN, J. H., 1981, The anatomy of the Galapagos Rift Limpet, *Neomphalus fretterae*. *Malacologia*, 21: 337–361.

GOLIKOV, A. & STAROBOGATOV, Y.I., 1975, Systematics of prosobranch gastropods. *Malacologia*, 15: 185–232.

GOULD, S. J., 1966, Notes on shell morphology and classification of the Siliquariidae (Gastropoda), the protoconch and slit of *Siliquaria squamata* Blainville. *American Museum Novitates*, 2263: 1–13.

GOULD, S. J., 1977, *Ontogeny and Phylogeny*. Harvard University Press, Cambridge, ix + 501 p.

GRAHAM, A., 1965, Observations on the anatomy

of some trochacean gastropods. *Bulletin of Marine Science*, 15: 202–210.

HARPER, J. A. & ROLLINS, H. A., 1982, Recognition of Monoplacophora and Gastropoda in the fossil record: a functional morphological look at the bellerophont controversy. *Third North American Paleontological Convention, Proceedings*, 1: 227–232.

HICKMAN, C. S., 1983, Ecological and phylogenetic implications of the unusual radula of *Laevinesta atlantica* (Mollusca: Gastropoda). *Veliger*, 25: 323–325, figs. 1-7.

HORNÝ, R. J., 1963, Lower Paleozoic Bellerophontina (Gastropoda) of Bohemia. *Sbornik Geologickych Ved Paleontologie*, 2: 57–164, 44 pl.

HORNÝ, R. J., 1965, *Cyrtolites* Conrad, 1838, and its position among the Monoplacophora (Mollusca). *Sbornik Národního Musea Ved Praze*, 21: 57–70.

HUDDLESTON, W. H., 1887–1896, A monograph of the British Jurassic Gasteropoda. Part 1. The Inferior Oolite Gasteropoda. *Palaeontographical Society, London,* 514 pp., 44 pl.

ILLINGWORTH, J. F., 1902, The anatomy of *Lucapina crenulata* Gray. *Zoologische Jahrbuchern, Abtheilung für Anatomie und Ontogenie der Thiere*, 16: 449–480, pl. 31–33.

KNIGHT, J. B., 1941, Paleozoic gastropod genotypes. *Geological Society of America Special Papers* 32, 510 p., 96 pl.

KNIGHT, J. B., 1947, Bellerophont muscle scars, *Journal of Paleontology,* 21: 264–267.

KNIGHT, J. B., 1952, Primitive fossil gastropods and their bearing on gastropod classification. *Smithsonian Miscellaneous Collections*, 117(13): 1–56.

KNIGHT, J. B., COX, L. R., KEEN, A. M., BATTEN, R. L., YOCHELSON, E. L. & ROBERTSON, R., 1960, Systematic descriptions (Archaeogastropoda), *In* MOORE, R. C., ed., *Treatise on Invertebrate Paleontology,* Part I, Mollusca 1: 169–310, Geological Society of America and University of Kansas Press.

KONINCK, L. G. DE, 1843, Description des animaux fossiles qui se trouvent dans le terrain carbonifère de Belgique. Liège, 651 p., 69 pl.

LEVER, J., 1979, On torsion in gastropods. *In* VAN DER SPOEL, S., VAN BRUGGEN, A. C. & LEVER, J., eds., *Pathways in Malacology*, Junk, The Hague, 5–23.

LINDSTRÖM, G., 1884, On the Silurian Gastropoda and Pteropoda of Gotland. *Kongliga Svenska Vetenskaps-Akademiens Handlingar,* 19(6): 1–250, 25 pl.

LINSLEY, R. M., 1977, Some "laws" of gastropod shell form. *Paleobiology,* 3: 196–206.

LINSLEY, R. M., 1978a, Shell form and the evolution of gastropods. *American Scientist*, 66: 432–441.

LINSLEY, R. M., 1978b, Locomotion rates and shell form in the Gastropoda. *Malacologia*, 17: 193–206.

MACCLINTOCK, C., 1963, Reclassification of gastropod *Proscutum* Fischer based on muscle scars and shell structure. *Journal of Paleontology,* 37: 141–156.

MACCLINTOCK, C., 1967, Shell structure of patelloid and bellerophontid gastropods (Mollusca). *Peabody Museum of Natural History, Yale University, Bulletin* 22: 1–140, 32 pl.

MARGOLIN, A. S., 1964, The mantle response of *Diodora aspera*. *Animal Behaviour*, 12: 187–194.

MCLEAN, J. H., 1981, The Galapagos Rift limpet *Neomphalus*: relevance to understanding the evolution of a major Paleozoic-Mesozoic radiation. *Malacologia*, 21: 291–336.

MEEK, F. B., 1866, Note on the affinities of the Bellerophontidae. *Chicago Academy of Sciences, Proceedings*, 1: 9–11.

MORTON, J. E. & YONGE, C. M., 1964, Classification and structure of the Mollusca. *In* WILBUR, K. M. & YONGE, C. M., eds., *Physiology of Mollusca*, 1: 1–58.

ODHNER, N. H., 1932, Zur Morphologie und Systematik der Fissurelliden. *Jenaische Zeitschrift für Naturwissenschaft*, 67: 292–309.

OWEN, G., 1958, Observations on the stomach and digestive gland of *Scutus breviculus* (Blainville). *Proceedings of the Malacological Society of London*, 33: 103–114.

PEEL, J. S., 1972, Observations on some Lower Palaeozoic tremanotiform Bellerophontacea (Gastropoda) from North America. *Palaeontology,* 15: 412–422, pl. 79.

PEEL, J. S., 1974, Systematics, ontogeny and functional morphology of Silurian trilobed bellerophontacean gastropods. *Bulletin of the Geological Society of Denmark,* 23: 231–261.

PEEL, J. S., 1975, A new Silurian gastropod from Wisconsin and the ecology of uncoiling in Palaeozoic gastropods. *Bulletin of the Geological Society of Denmark,* 24: 211–221.

PEEL, J. S., 1976, Musculature and systematic position of *Megalomphala taenia* (Bellerophontacea; Gastropoda) from the Silurian of Gotland. *Bulletin of the Geological Society of Denmark*, 25: 49–55.

PEEL, J. S., 1980, A new Silurian retractile monoplacophoran and the origin of the gastropods. *Proceedings of the Geological Association,* 91: 91–97.

PILSBRY, H. A. & MCGINTY, T. L., 1952, Notes on *Nesta (Laevinesta) atlantica*, a Floridan fissurellid mollusk. *Nautilus*, 66: 1–3.

POJETA, J. & RUNNEGAR, B., 1976, The paleontology of rostroconch mollusks and the early history of the phylum Mollusca. [*United States] Geological Survey Professional Paper* 968: 1–88.

RANDLES, W. B., 1905, Some observations on the anatomy and affinity of the Trochidae. *Quarterly Journal of Microscopical Science,* 78: 33–78, pl. 4–6.

RISBEC, J., 1939, Recherches anatomiques sur les Prosobranches de Nouvelle-Calédonie. *Annales des Sciences Naturelles, Zoologie,* ser. 11, 2: 235–299.

ROBERTSON, R., 1964, The hyperstrophic larval

shells of the Architectonicidae. *Annual Reports for 1963 of the American Malacological Union:* 11–12.

ROLLINS, H. B., 1967, *The phylogeny and functional morphology of the Knightitinae, Carinaropsinae, and Praematuratropidae (Gastropoda, Bellerophontacea).* Columbia University, Ph.D. thesis, 197 p.

ROLLINS, H. B., 1969, The taxonomic position of *Cyrtonella mitella* (Hall) (Mollusca, Monoplacophora). *Journal of Paleontology,* 43: 136–140.

ROLLINS, H. B. & BATTEN, R., 1968, A sinus-bearing monoplacophoran and its role in the classification of primitive molluscs. *Paleontology,* 11: 132–140.

RUNNEGAR, B., 1981, Muscle scars, shell form and torsion in Cambrian and Ordovician univalved molluscs. *Lethaia,* 14: 311–322.

RUNNEGAR, B. & JELL, P. A., 1976, Australian Middle Cambrian molluscs and their bearing on early molluscan evolution. *Alcheringa,* 1: 109–138.

RUNNEGAR, B. & POJETA, J., 1974, Molluscan phylogeny: the paleontological viewpoint. *Science,* 186: 311–317.

SALVINI-PLAWEN, L. v., 1980, A reconsideration of systematics in the Mollusca (phylogeny and higher classification). *Malacologia,* 19: 249–278.

SALVINI-PLAWEN, L. v., 1981, On the origin and evolution of the Mollusca. In *Origine dei Grandi Phyla dei Metazoi,* Convegno Internazionale, Roma, 7–9 Maggio 1979, Accademia Nazionale dei Lincei, Atti dei Convegni Lincei, 49: 235–293.

SCHEPMAN, M. M., 1908, The Prosobranchia of the Siboga Expedition. Part 1. Rhipidoglossa and Docoglossa. *Siboga-Expeditie,* 49a: 1–107, pl. 1–9.

STASEK, C. R. & MCWILLIAMS, W. R., 1973, The comparative morphology and evolution of the molluscan mantle edge. *Veliger,* 16: 1–19.

STAROBOGATOV, Y. I., 1970, Systematics of Early Paleozoic Monoplacophora. *Paleontological Journal,* 4: 293–302. [Translated from Russian, *Paleont. Zhur.* 1970(3): 6–17.]

TERMIER, G. & TERMIER, H., 1968, Evolution et paléontologie des Gastéropodes. *In* GRASSÉ, P. P., ed., *Traité de Zoologie, Anatomie, Systématique, Biologie,* V (III), 284–923, Masson, Paris.

THIELE, J., 1929, Handbuch der systematischen Weichtierkunde. Erster Teil, Loricata, Gastropoda. I. Prosobranchia (Vorderkiemer). Fischer, Jena, 376 p.

TOBLER, M., 1902, Zur Anatomie von *Parmophorus intermedius* Reeve. *Jenaische Zeitschrift für Naturwissenschaft,* 36: 229–274, pl. 13–15.

VAYSSIÈRE, A., 1894, Étude zoologique de la *Scissurella costata,* var. *laevigata. Journal de Conchyliologie,* ser. 3, 42: 18–29, pl. 2.

WENZ, W., 1940, Ursprung und frühe Stammesgeschichte der Gastropoden. *Archiv für Molluskenkunde,* 72: 1–10.

WOODWARD, M. F., 1901, The anatomy of *Pleurotomaria beyrichii* Hilg. *Quarterly Journal of Microscopical Science,* 44: 215–268, pl. 13–16.

YOCHELSON, E. L., 1960, Permian Gastropoda of the southwestern United States. 3. Bellerophontacea and Patellacea. *Bulletin of the American Museum of Natural History,* 119: 205–294, pl. 46–57.

YOCHELSON, E. L., 1967, Quo vadis, *Bellerophon? In* TEICHERT, C. & YOCHELSON, E. L., eds., *Essays in Paleontology and Stratigraphy,* University of Kansas Press, Lawrence, Kansas, 141–161.

YOCHELSON, E. L., 1978, An alternative approach to the interpretation of the phylogeny of ancient mollusks. *Malacologia,* 17: 165–191.

YOCHELSON, E. L., 1979, Early radiation of Mollusca and mollusc-like groups. *In* HOUSE, M. R., ed., *The Origin of Major Invertebrate Groups,* Systematics Association Special Volume No. 12, Academic Press, London: 323–358, pl. 4–6.

YONGE, C. M., 1947, The pallial organs in the aspidobranch Gastropoda and their evolution throughout the Mollusca. *Philosophical Transactions of the Royal Society of London,* ser. B, 232: 443–517, 1 pl.

ZIEGENHORN, A. & THIEM, H., 1926, Beiträge zur Systematik und Anatomie der Fissurellen. *Jenaische Zeitschrift für Naturwissenschaft,* 62: 1–78, pl. 1–6.

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A REVIEW OF THE GENERA OF THE IRAVADIIDAE (GASTROPODA: RISSOACEA) WITH AN ASSESSMENT OF THE RELATIONSHIPS OF THE FAMILY

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ABSTRACT

The family Iravadiidae is defined and shown to have a close relationship with the Hydrobiidae, not the Rissoidae as previously thought. Nine genera and five subgenera (of *Iravadia*) are provisionally recognised. Iravadiids live mainly in the Indo-West Pacific region in brackish-waters, or are marine. Seven new species, two new genera and one new subgenus are described.

The Iravadiidae have a slit-like ventral opening to the pallial genital duct in the female. The bursal duct and, with one exception, the bursa copulatrix, is entirely in the pallial part of the genital tract, not posterior to the pallial cavity as in the Hydrobiidae and the Rissoidae. It also differs from these families in having a rudimentary oesophageal gland. In most other respects the Iravadiidae resemble the Hydrobiidae. The family is shown to have a wide range of shell, radular and opercular forms.

Previously-named genera recognised as valid in the family are *Iravadia* (with *Fluviocingula, Fairbankia* and *Pseudonoba* as subgenera), *Rissopsis, Hyala, Ceratia, Nozeba, Chevallieria* and *Rhombostoma*.

Key words: Gastropoda; Rissoacea; Iravadiidae; taxonomy; systematics; anatomy; mangroves.

INTRODUCTION

Several of the genera considered here to be iravadiids have been regarded as members of the Rissoidae (Cossmann, 1921; Thiele, 1929; Wenz, 1939; Coan, 1964; Ponder, 1967) by most revisers. This revision has grown out of a survey of the genera of the Rissoidae currently being undertaken by the writer. During this work several genera, which previously had no suspected phyletic relationships, were tentatively grouped together principally because all possessed a peculiar, flat-topped, smooth protoconch with a very small initial whorl. Examination of the radula and anatomy of representative species has confirmed their relationship and suggested their separation into a family within the Rissoacea. This family grouping shares shell characters with the Rissoidae but in many anatomical characters resembles the Hydrobiidae.

In the last major revision of the mesogastropods (Wenz, 1939), the genera here regarded as iravadiids were included in 6 different families (Iravadiidae, Micromelaniidae, Rissoidae, Aclididae, Thiaridae and Eulimidae). The purpose of this paper is to outline the distinctive features of the Iravadiidae, define the genera and discuss the relationships of the family.

The genus *Iravadia* Blanford was proposed for a small, spirally-sculptured, brackish-water gastropod from India. The usage of this name has been restricted to a few species of similar appearance, mainly from estuarine habitats. Thiele (1928) proposed new family-group names for *Iravadia* and for *Fairbankia* Stoliczka, both genera being based on species found in mangroves in southern Asia. Brandt (1968) showed that, on the basis of their shells, radulae, opercula and external features of the head-foot, *Fairbankia* and *Iravadia* are closely related. This relationship has been confirmed by anatomical studies during this work.

Johansson (1950) described the female reproductive system of a European marine species, *Hyala vitrea* (Montagu), and showed it to have an anterior bursa copulatrix and a slit-like opening near the posterior end of the glandular pallial duct. Golikov & Starobogatov

(1975) considered these differences sufficiently great to create a new family for *Hyala*.

Anatomical work on species of *Iravadia* s.l. shows that their anatomy is similar to that of *Hyala vitrea* and that the *Iravadia-Hyala* group can be distinguished anatomically from the Hydrobiidae and Rissoidae.

MATERIALS AND METHODS

The specimens used in this survey are housed in several museums (see list of abbreviations), although the majority of the observations are based on material held in The Australian Museum. Scanning electron micrographs were obtained from material prepared as described by Ponder & Yoo (1976). Anatomical work was carried out on material fixed in Bouin's fixative or 5%–10% neutral formalin. This was sectioned at 5–6 μm and stained with Mallory's triple stain.

The generic diagnoses are intended to encompass all species included in the genus but the detail of opercular, radular and head-foot description is based only on those species for which the information is available. These species are cited in the diagnoses except when the genus is monotypic or when only the type-species has been examined.

The species listed under "Distribution" are principally those examined and confirmed as belonging to the genus. Whenever possible, names of other species probably belonging to the genus are also given. A list of material examined is given under each genus to indicate the range available to the writer and to assist future confirmation.

ABBREVIATIONS USED IN FIGURES

ag	albumen gland
agr	accessory groove
ass	anterior sperm sac
bc	bursa copulatrix
bd	duct of bursa copulatrix
cg	capsule gland
co	coiling part of oviduct
df	dorsal fold
gle	glandular epithelium
gp	gonopore
hpg	hypobranchial gland
ibc	opening of bursa copulatrix to capsule gland
lf	longitudinal fold
log	lower oviduct gland
mf	muscular fold
obc	pallial opening of bursa copulatrix
og	rudimentary oesophageal gland
p	prostate gland
pd	pallial duct from prostate gland
pm	posterior limit of pallial cavity
pvd	pallial vas deferens
r	rectum
rgd	reno-gonidial duct
ro	renal oviduct
sg	sperm groove
sr	seminal receptacle
sv	seminal vesicle
uog	upper oviduct gland
vc	ventral channel
vd	vas deferens
vf	ventral (outer) fold of sperm groove

MUSEUM ABBREVIATIONS

AIM	Auckland Institute and Museum, New Zealand.
AMS	Australian Museum, Sydney, Australia.
ANSP	Academy of Natural Sciences of Philadelphia, U.S.A.
BMNH	British Museum (Natural History), London, U.K.
GIT	Geological Institute, University of Tokyo, Japan.
GNHM	Natural History Museum, Genoa, Italy.
HUM	Humboldt Universität Museum, E. Berlin, E. Germany
IRSNB	Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium.
NHMP	Muséum National d'Histoire Naturelle, Paris, France.
NM	Natal Museum, Pietermaritzburg, South Africa.
NMNZ	National Museum, Wellington, New Zealand.
NMV	National Museum of Victoria, Melbourne, Australia.
NMW	National Museum of Wales, Cardiff, U.K.
NSMT	National Science Museum, Tokyo, Japan.
SAM	South Australian Museum, Adelaide, Australia.
SMF	Senckenberg Museum, Frankfurt, W. Germany.
TGM	Geological Museum, Turin, Italy.
USBF	United States Bureau of Fisheries.
USNM	National Museum of Natural History, Washington, D.C., U.S.A.
ZMR	Zoological Museum, Rome, Italy.

TAXONOMY

Family IRAVADIIDAE Thiele, 1928

Synonyms: Fairbankiinae Thiele, 1928; Hyalidae Golikov & Starobogatov, 1975.

Diagnosis. Shell narrowly-conic to ovate-conic, usually solid, lacking inner chitinous shell layer, usually nonumbilicate, smooth or with spiral sculpture predominant, axial sculpture present as growth-lines, lamellae or, rarely, ribs. Aperture oval, usually weakly to distinctly angled anteriorly and posteriorly; varix on outer lip broad and strong to absent. Protoconch small, smooth, of about 1¾–2½ convex whorls, planorbid (coiled in one plane) to depressed dome-shaped; first whorl minute, terminated by a distinct varix in some species. Periostracum sometimes well developed, rarely bearing processes. Head-foot often pigmented, cephalic tentacles long, slender, usually with a few stationary cilia, sometimes with colour bands; eyes at their outer bases. Snout of moderate length, usually bilobed. Foot usually with anterior edge indented and expanded laterally. Posterior end of foot pointed, rounded, slightly indented or deeply bifurcate. No posterior pedal mucous gland except for a rich supply of subepithelial glands in some species; anterior pedal gland distinct, confined to pedal haemocoel. Foot lacking a metapodial tentacle in most species. No pallial tentacle in most species. Radula taenioglossan, central teeth with zero to four pairs of basal denticles. Operculum with eccentric, submarginal or marginal nucleus. Penis short, thick, bent double or partially coiled when at rest; with a single distal or lateral opening and with accessory, often glandular, processes on a flattened, broad distal end in most species. Female genital duct comprises a short, simple renal oviduct opening directly to albumen gland. One or two seminal receptacles (usually one) open at point where renal oviduct joins albumen gland. Capsule gland with ventral gonopore variable in position and length; usually opening slit-like, either posterior, in middle section, subterminal or (in one species) most of ventral side of anterior two-thirds of gland open. Bursa copulatrix variable in position, entirely pallial in most species; bursal duct, when present, pallial (i.e., opens to capsule gland) or bursa opens directly to pallial cavity by vertical opening. A sperm sac anterior to gonopore developed in some species. Long dorsal folds in anterior part of oesophagus; rudimentary oesophageal gland present. Crystalline style present in stomach. Nervous system similar to that of Hydrobiidae.

Remarks. The family name Iravadiidae was given precedence over Fairbankiidae by Brandt (1968) and this is upheld here as the action of the first reviser. Starobogatov (1970) has used Fairbankiidae to include both *Iravadia* and *Fairbankia*.

The main characters separating the Iravadiidae from the two closest families, the Rissoidae and the Hydrobiidae, are given in Table 1.

General Anatomical Description

No attempt has been made to fully describe the anatomy of any of the species examined. The species examined anatomically are *Iravadia ornata* (Blanford), *Iravadia quadrasi* (Boettger), *Iravadia (Fairbankia) bombayana* (Stoliczka), *I. (F.) australis* Hedley, *Iravadia (Pseudomerelina* nov.) *mahimensis* (Melvill), *Nozeba topaziaca* (Hedley), and *Hyala vitrea* (Montagu).

The Pallial Cavity. There is a well-developed ctenidium reaching to the posterior end of the pallial cavity and a conspicuous osphradium half to two-thirds the length of the ctenidium. The osphradium consists of a

TABLE 1. The main features separating the Iravadiidae, Hydrobiidae and Rissoidae.

	Iravadiidae	Hydrobiidae (s.l.)	Rissoidae
Shell			
Aperture	With weak to strong anterior channel	Usually without anterior channel	With or without anterior channel
Sculpture	Smooth or spiral predominant	Smooth, spiral or axial predominant	Smooth, spiral or axial predominant
Protoconch	Planorbid to depressed-dome-shaped, about 2 whorls, smooth	Dome-shaped, about 1½ whorls, often pitted	Dome-shaped to subconical, 1½ to about 3 whorls, sculpture variable, rarely smooth

TABLE 1 (Continued)

	Iravadiidae	Hydrobiidae (s.l.)	Rissoidae
Operculum	Oval, nucleus eccentric or marginal. Without peg and calcareous material	Oval to circular, nucleus eccentric to central. Sometimes with calcareous peg or smear	Oval, nucleus eccentric. Sometimes with horny peg; without calcareous material
Radula			
Central teeth	0–4 pairs of basal cusps, rudimentary in some species	0 to several pairs of basal cusps	1–3 pairs of basal cusps
Head-foot			
Cephalic tentacles	Long, ciliated, pigmented to unpigmented	Long, ciliated or smooth, pigmented or unpigmented	Long to moderately short, usually ciliated, rarely pigmented
Pallial tentacle(s)	Rudimentary or absent	Rudimentary or absent	Present or absent
Metapodial tentacle(s)	Usually absent	Usually absent	Often present
Posterior pedal mucous gland	Absent	Absent	Present or absent
Reproductive systems Ventral wall of pallial oviduct with fold enclosing ventral channel	Yes	Yes	No
Ventral wall of pallial oviduct	Open, or closed except for posterior, subterminal or median opening	Closed except for small terminal or subterminal opening	Closed (open in *Merelina* Iredale), except for terminal or subterminal opening
Glandular oviduct	Single gland	Single gland	Two glands
Anterior sperm sac	Present or absent	Absent	Usually absent
Bursa copulatrix	Anterior or posterior, within roof of pallial cavity (with one exception)	Posterior, within visceral mass	Posterior, within visceral mass
Renal oviduct	Renal oviduct opens directly to albumen gland	Renal oviduct opens to narrow oviducal coil	Renal oviduct opens to upper oviduct gland or (rarely) oviducal coil
Penis	With swollen distal end and accessory glandular structures	Simple or with accessory glandular structures	Usually simple; *Rissoina* with swollen distal end and accessory glandular structures
Prostate gland	Half within roof of pallial cavity	Half within roof of pallial cavity or (rarely) entirely behind cavity	None, or half to entirely within roof of pallial cavity; rarely behind cavity
Digestive system			
Mid-oesophagus	With long dorsal folds; with rudimentary oesophageal gland	With long dorsal folds; no oesophageal gland	With short dorsal folds; no oesophageal gland
Spherules in secretory cells in digestive gland	Absent	Present	Present
Nervous system			
Left pleural- and suboesophageal ganglia	Abutting	Fused or with short connective	Very short connective to abutting
Right pleural-supraoesophageal ganglia	With long connective	With long to short connective	With moderately long connective

FIG. 1. Head-foot of some species of Iravadiidae drawn from living material. A. *Iravadia (Fairbankia) australis* (Hedley). Magnetic Island, Queensland, Australia. B. *Iravadia (Iravadia) quadrasi* (Boettger). Darwin, Northern Territory, Australia. C. *Iravadia (Pseudomerelina) mahimensis* (Melvill). Maningrida, Northern Territory, Australia. D. *Iravadia (Fairbankia) bombayana* (Stoliczka). Sembawang, Singapore. E. *Iravadia (Iravadia) ornata* (Blanford). Deep Bay, New Territories, Hong Kong. F. *Iravadia (Fluviocingula) resima* (Laseron). Darwin, Northern Territory, Australia. G. *Liroceratia sulcata* (Boettger). Taurama, near Port Moresby, Papua New Guinea. H. *Iravadia (Pseudonoba) bella* (Adams). Magnetic Island, Queensland, Australia. I. *Nozeba topaziaca* (Hedley). Port Hacking, New South Wales, Australia. Scales = 0.3 mm.

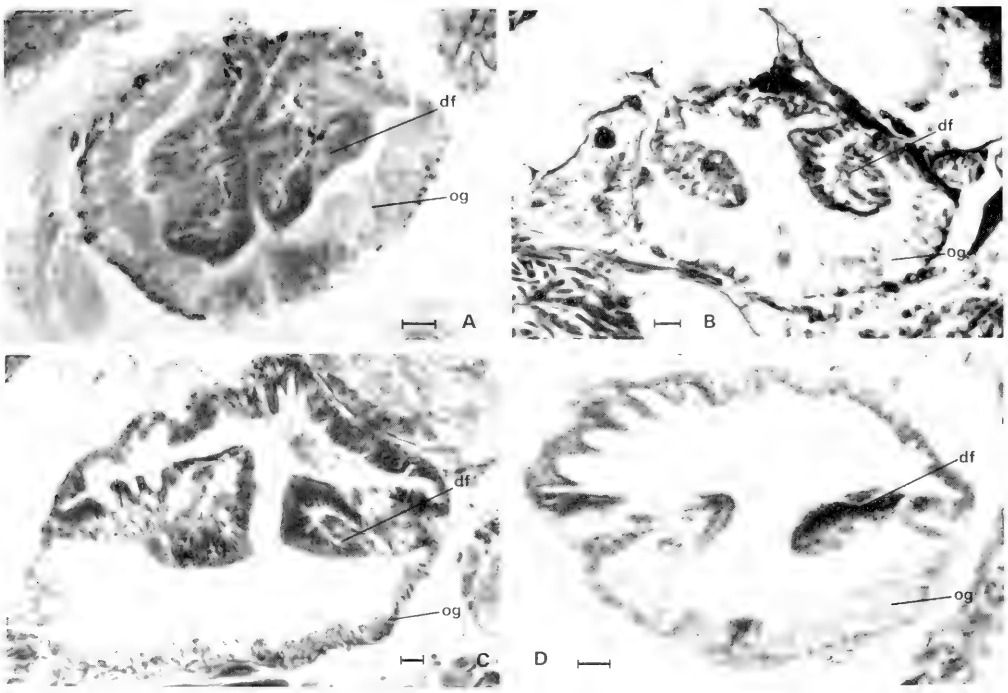

FIG. 2. Transverse sections of the mid-oesophagus of four species of the Iravadiidae. A. *Nozeba topaziaca* (Hedley). B. *Hyala vitrea* (Montagu). C. *Iravadia (Iravadia) ornata* (Blanford). D. *Iravadia (Fairbankia) australis* (Hedley). Scales = 0.01 mm.

broad, central sensory area bordered by narrow, ciliated ridges. A hypobranchial gland is also present.

Alimentary Canal. Generally similar to that of the Hydrobiidae. The buccal mass is large and there is a pair of jaws composed of chitinous rodlets. The salivary glands are tubular and pass dorsal to the circumoesophageal nerve ring. The anterior part of the oesophagus is short to rather long, with a pair of long dorsal folds (Fig. 2, df) that coil upwards in the larger species (*Iravadia* spp., *I. (Fairbankia)* spp.) (Fig. 2C, D) but are simple in *Nozeba topaziaca* (Fig. 2A), *Hyala vitrea* (Fig. 2B) and *I. (Pseudomerelina) mahimensis*. All of these species have glandular tissue in the ventral wall of the mid-oesophagus (Fig. 2, og). This glandular epithelium is composed of irregular columnar cells and is interpreted as a remnant of an oesophageal gland. Comparison of the same region in rissoids and hydrobiids shows a simple, ciliated epithelium in which are scattered only a few gland cells. The stomach has a crystalline style in a well-developed style sac. The rectum is looped in the dorsal wall of the mantle cavity and typically has the faecal pellets packed sideways. The digestive gland has two types of digestive cells and the excretory cells do not contain large refractive granules.

Female Reproductive System. This system was examined in detail, mainly by serial sections. Because of the brittle nature of the oviduct glands and the limited amount of material, some observations could not be confirmed and others are doubtful for particular species. The single female sectioned of *I. ornata* was not fully mature.

The narrow, thin-walled upper oviduct opens into a slightly thicker, ciliated, nonmuscular, short renal oviduct. No gonopericardial or reno-gonidial duct was observed in any species except *I. ornata*, where there is a short, ciliated reno-gonidial duct. The glandular part of the oviduct consists of a posterior albumen gland (Figs. 4–7, ag) which partially displaces the kidney and which is continuous with the capsule gland lying in the roof of the pallial cavity. A seminal receptacle lies along-

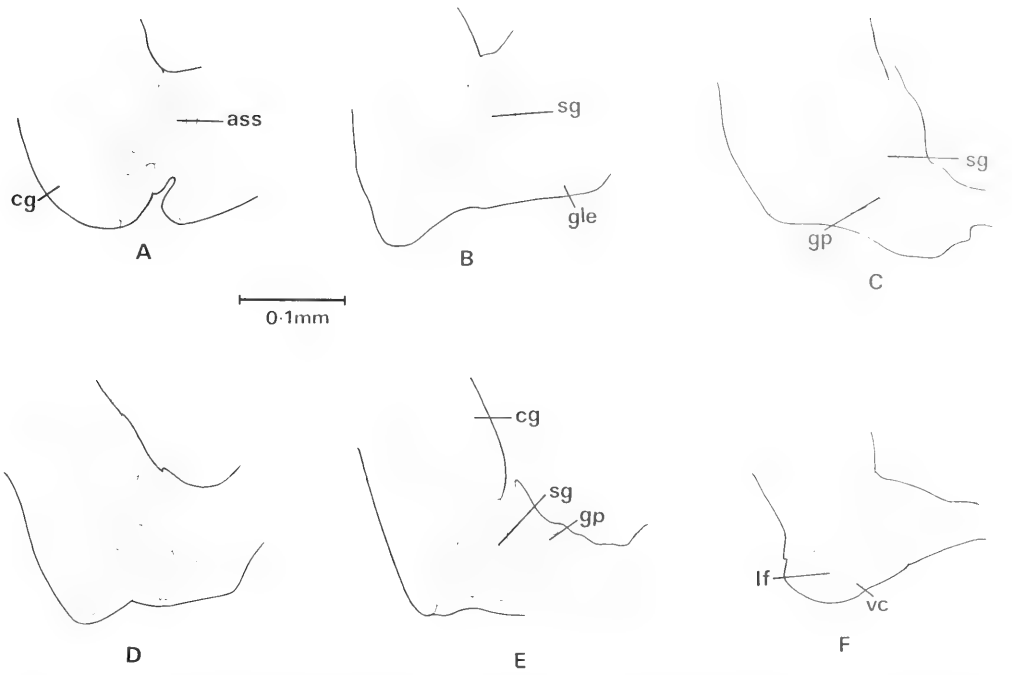

FIG. 3. A–F. Transverse sections through the ventral part of the female pallial genital duct of *Hyala vitrea* from near the anterior end to near the posterior end. All figures to same scale.

side the albumen gland and is partly embedded in it. *Hyala* has one (?) or two seminal receptacles and *Iravadia (Pseudomerelina) mahimensis* appears to have two. The other species appear to have only one (Figs. 4–7, sr). The seminal receptacle(s), together with a renal oviduct, opens into the oviduct just posterior to the capsule gland. This gland (Figs. 3–7, cg) opens to the pallial cavity by a long to short, slit-like ventral opening (gp) or gonopore. In *Nozeba topaziaca* and *Hyala vitrea* the oviduct aperture is in the posterior part of the capsule gland and the anterior part of the gland is blind (Fig. 21B). The oviduct opening extends along all of the anterior two-thirds of the ventral side of the capsule gland in *I. (Fairbankia) bombayana* (Fig. 7D) but is short and placed in the anterior part of the gland in *I. ornata* (Fig. 21G), *I. (Pseudomerelina) mahimensis* (Fig. 5) and *I. (Fairbankia) australis*) (Fig. 6). It is long and in the middle of the gland in *I. quadrasi* (Fig. 4). There is a deep sperm groove (sg) on the lower, inner part of the inner (left) ventral wall of the capsule gland in the vicinity of the pallial opening in *I. (F.) australis, I. quadrasi* (Fig. 4B–D), *I. (P.) mahimensis* (Fig. 5B), *N. topaziaca* and *H. vitrea* (Fig. 3B–E). This gutter runs forward and closes over to form an anterior sperm sac (Figs. 3–6, ass) which appears to function as a bursa copulatrix. In *I. (F.) bombayana* there is a sperm sac on the outer (left) side of the inner (left) wall of the posteror third of the capsule gland and it is wholly within the roof of the pallial cavity (Fig. 7, bc). This sac opens by way of a vertical slit (Fig. 7, obc), the outer edge of which represents the outer fold of the sperm groove, and its internal walls are heavily folded. In *I. (F.) australis* (Fig. 6) there is a similar sperm sac (bc), the opening (obc) of which lies in the anterior second quarter of the capsule gland, but there is also a small anterior sperm sac (ass) anterior to the oviducal opening. To enable these sperm sacs to be distinguished, the structure external to the capsule gland seen in *I. (Fairbankia)* species is hereafter called the bursa copulatrix and the sperm sac anterior to the oviducal opening is referred to as the anterior sperm sac. Both structures appear to function as a bursa copulatrix but no evidence of sperm ingestion was observed in sections of either structure. They both store sperm, although the bursa copulatrix was empty in most speci-

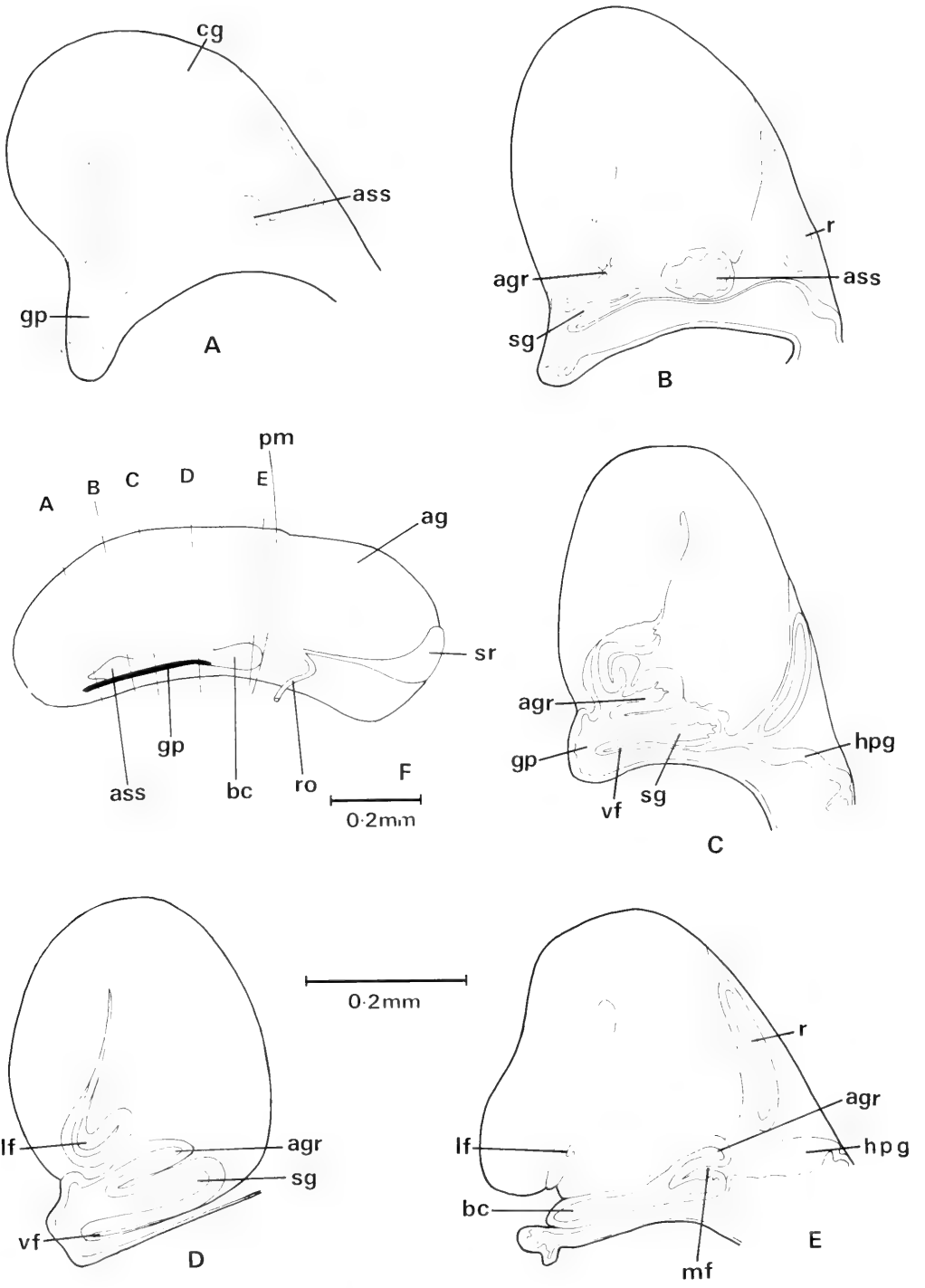

FIG. 4. Female reproductive system of *Iravadia (Iravadia) quadrasi*. A–E. Sections through the pallial genitalia at the positions marked on Fig. F. F. Lateral view of female genital system (excluding upper oviduct and ovary). Figs. A–E to same scale.

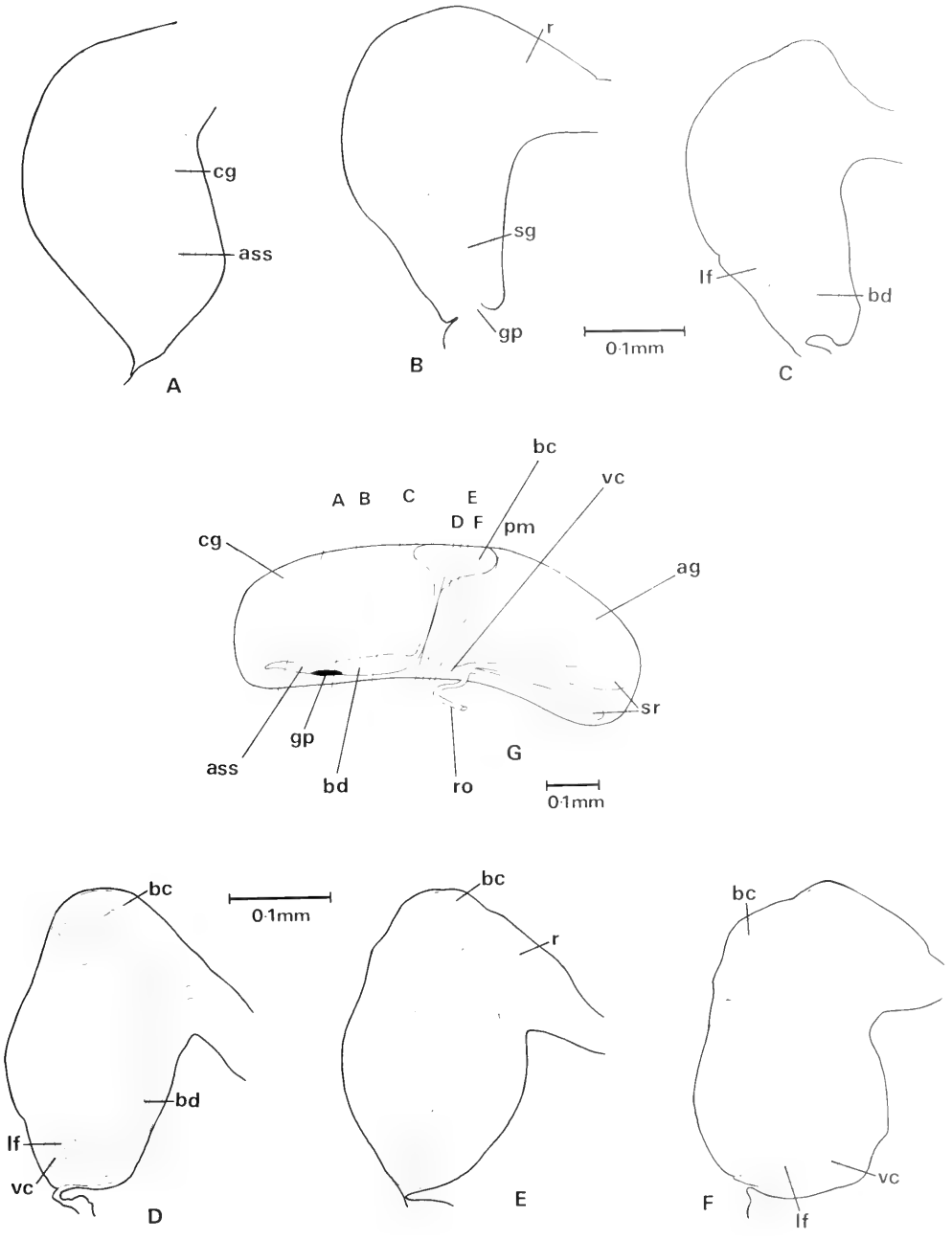

FIG. 5. Female reproductive system of *Iravadia (Pseudomerelina) mahimensis*. A–F. Transverse sections of the pallial genitalia at the positions marked on Fig. G. G. Lateral view of female reproductive system, excluding the upper oviduct and ovary. Figs. A–F to same scale.

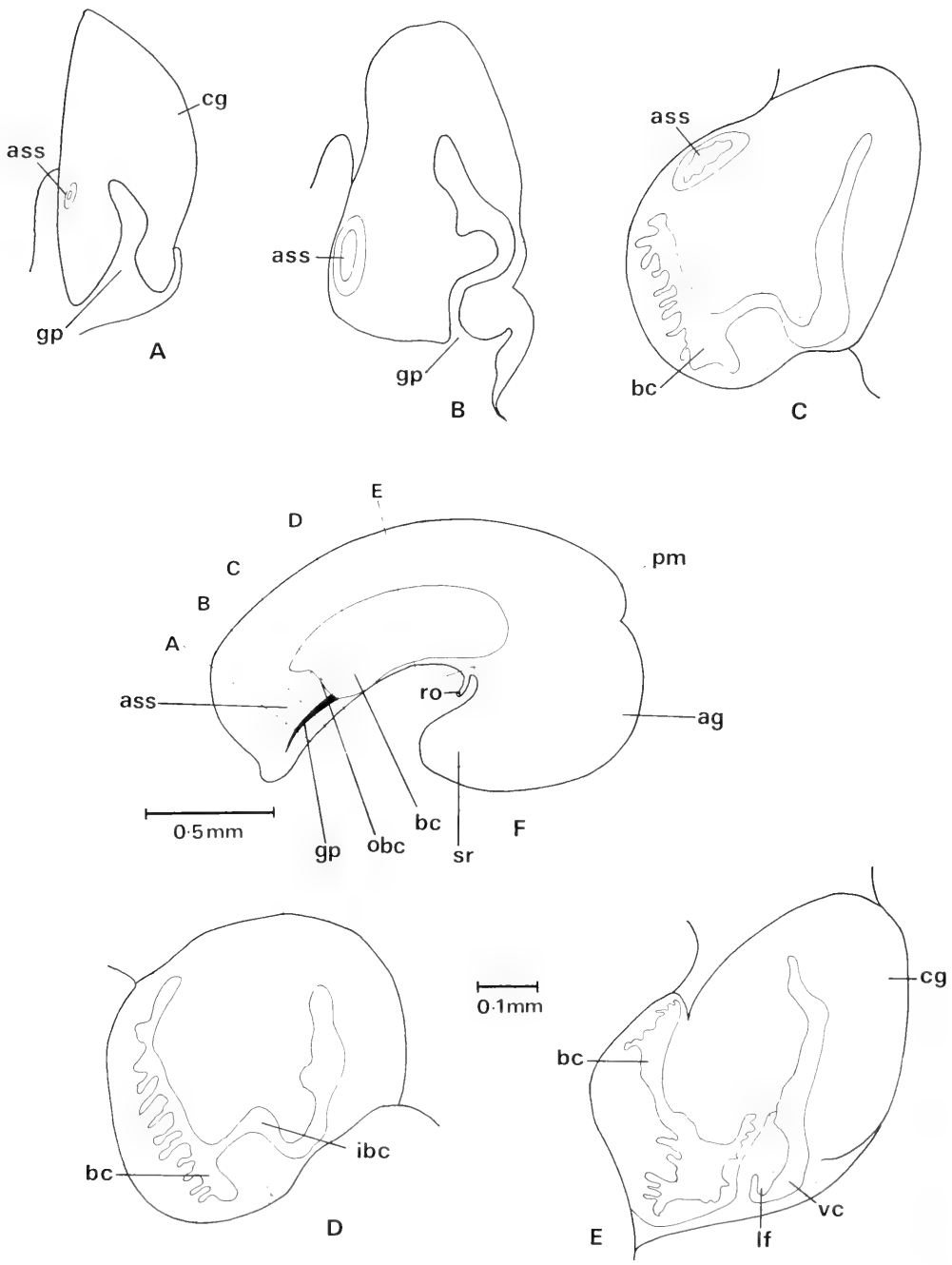

FIG. 6. Female reproductive system of *Iravadia (Fairbankia) australis*. A–E. Section through the pallial genitalia at the positions marked in Fig. F. F. Lateral view of female reproductive system, excluding the upper oviduct and ovary. Figs. A–E to same scale.

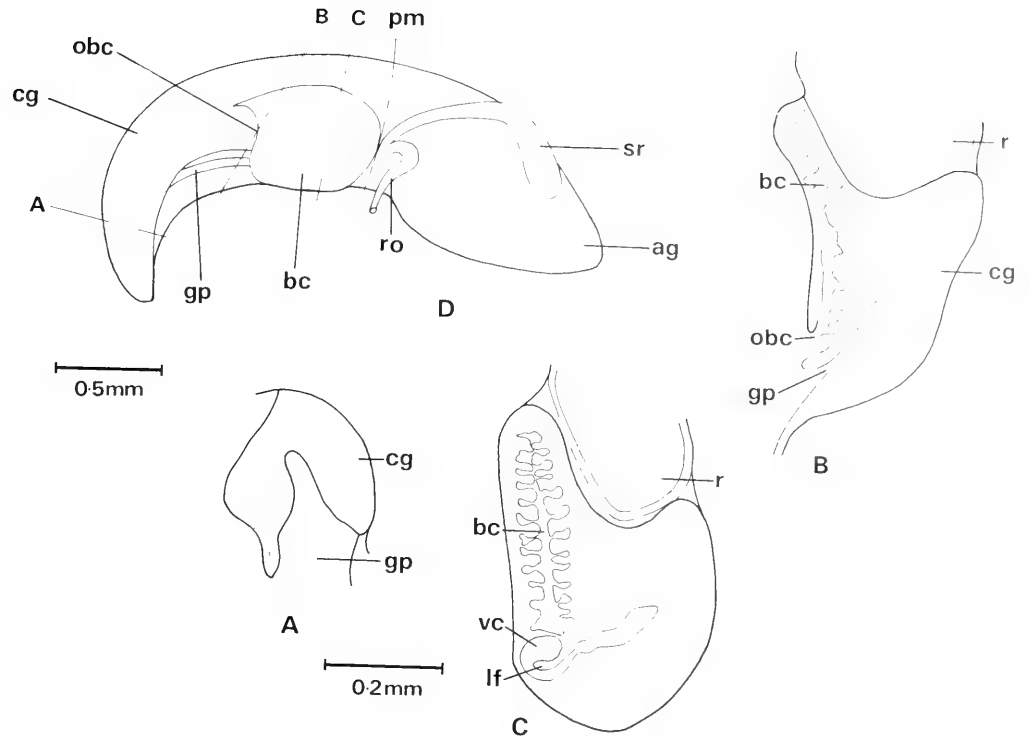

FIG. 7. Female reproductive system of *Iravadia (Fairbankia) bombayana*. A–C. Sections through the pallial genitalia at the position marked in Fig. D. D. Lateral view of female genital system, excluding the upper oviduct and ovary. Figs. A–C to same scale.

mens sectioned, suggesting a very temporary storage function.

In the two species of *Iravadia (Fairbankia)* the bursa copulatrix is open laterally to the capsule gland for a short distance (Fig. 6, ibc) and is then separated from it and becomes a blind pocket (Fig. 6E, 7C). The seminal receptacle (sr) opens into the posterior end of the ventral channel. The posterior section of the ventral channel has, as in all the species examined, a ciliated fold (lf) similar to that seen in the Hydrobiidae (see Discussion). *Iravadia quadrasi* (Fig. 4) has a ventral bursa copulatrix (bc) behind the oviducal opening extending back to the end of the pallial cavity. This structure is formed from the closure of the ventral (outer) fold of the sperm groove (vf), but the pallial opening of the capsule gland is closed slightly in front by the merger of the inner ventral edge of the ventral channel of the capsule gland and the upper edge of an accessory fold on the upper side of the sperm groove (Fig. 4C, D). This accessory fold is the dorsal edge of a glandular groove (Fig. 4, agr) containing distinctive, blue-staining, cuboidal gland cells in its anterior section. Anteriorly, the groove is blind and merges with the capsule gland after forming a tubular structure. Posteriorly, it continues into the bursa copulatrix where its epithelium is similar to that of the remainder of the bursa. The prominent muscular fold (Fig. 4E, mf) separating the sperm groove and the groove dorsal to it, continues through the bursa. It was not possible to determine from the available material if the fold continued the full length of the bursa. In *I. (Pseudomerelina) mahimensis* (Fig. 5) the duct of the bursa copulatrix (bd) has been formed by the closure of the sperm groove just behind the opening to the capsule gland (Fig. 5, B–C). The tubular bursal duct runs posteriorly and then dorsally into the dorsally placed bursa copulatrix (bc) which lies above the posterior end of the capsule gland (Fig. 5, D–G). The type-species of *Iravadia, I. ornata* (Fig. 21G), shows a similar arrangement to that seen in the Hydrobiidae in possessing an anterior

oviducal opening, a ciliated fold in the ventral channel of the capsule gland and no anterior sperm sac. The bursal duct opens immediately behind the short, slit-like, muscular, subterminal opening and runs dorsally over the inner wall of the gland to open to the long, tubular bursa which lies latero-dorsally on the outer (right) side of the capsule gland and the anterior half of the albumen gland. Thus the bursa extends behind the posterior end of the

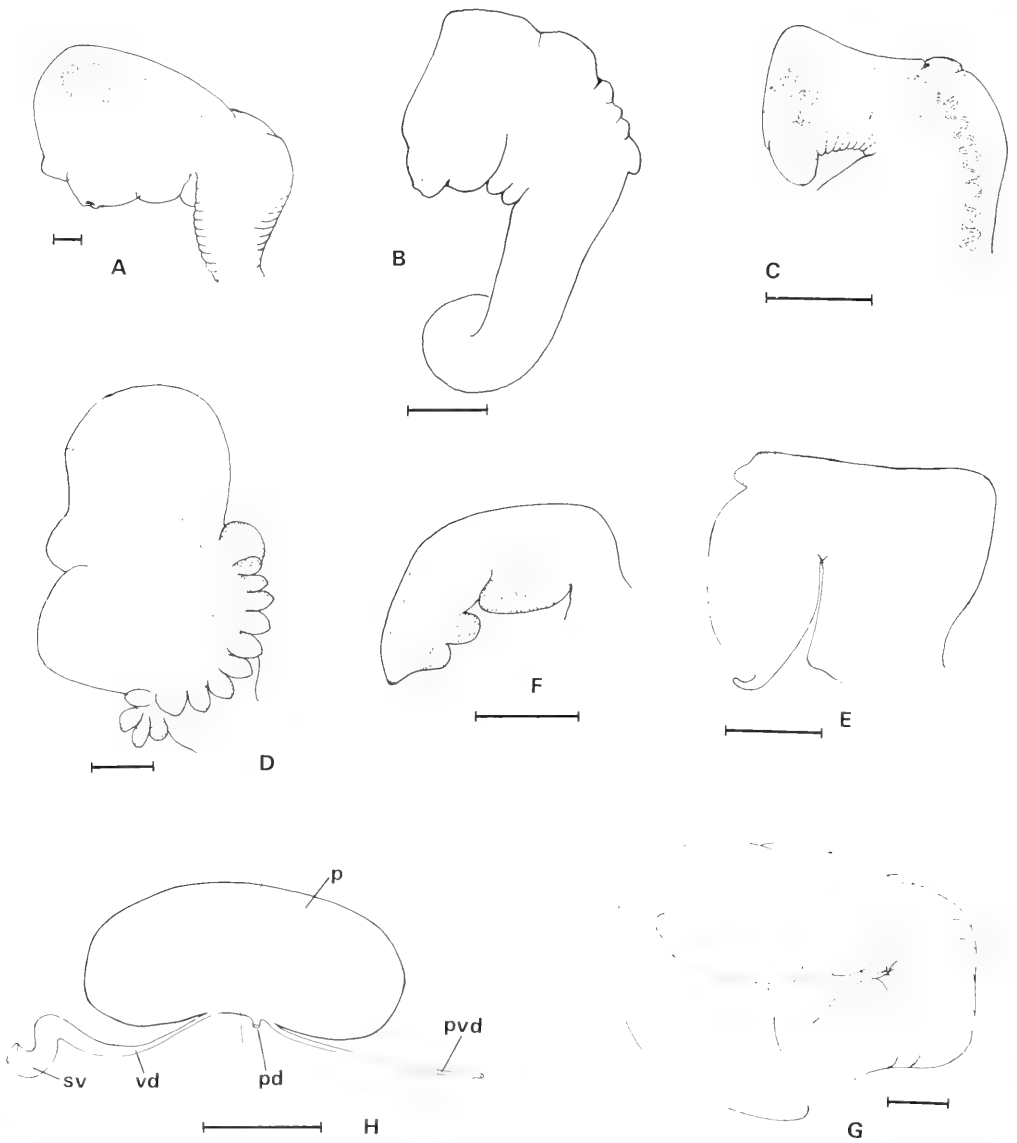

FIG. 8. A–G. Penes of some species of Iravadiidae. In Figs. C–F glandular areas are represented by stipple. A. *Iravadia (Iravadia) ornata* (Blanford). Deep Bay, New Territories, Hong Kong. B. *Iravadia (Iravadia) quadrasi* (Boettger). Hervey Bay, Queensland, Australia. C. *Iravadia (Pseudomerelina) mahimensis* (Melvill). Magnetic Island, Queensland, Australia. D. *Iravadia (Fairbankia) australis* (Hedley). Magnetic Island, Queensland, Australia. E. *Hyala vitrea* (Montagu). Ten km W of Strömstad, W Sweden, 35 m. F. *Nozeba topaziaca* (Hedley). Port Hacking, New South Wales, Australia. G. *Iravadia (Fairbankia) bombayana* (Stoliczka). Sembawang Estuary, Singapore. H. Diagrammatic representation of the prostate gland and vas deferens of *Iravadia (Iravadia) ornata* (Blanford). Scales = 0.2 mm.

pallial cavity in this species. A thin-walled vestibular region may function as an anterior sperm sac.

Male Reproductive System. Examined in *Iravadia ornata, I. quadrasi, I. (Fairbankia) bombayana, I. (F.) australis, I. (Pseudomerelina) mahimensis* and *Hyala vitrea.*

The vas deferens forms a long, coiled seminal vesicle (Fig. 8H, sv) and the renal vas deferens is short and ciliated. The bean-shaped prostate gland (p) lies half within the pallial roof and has a median lumen except in a short middle section which is thin-walled ventrally. This section receives the renal vas deferens behind the posterior pallial wall and the pallial vas deferens (pvd) emerges in front of this wall. There is a very short, ciliated duct (pd) in *I. (Pseudomerelina) mahimensis* and *I. ornata* which emerges next to the junction of the prostate gland and the pallial vas deferens, and opens to the pallial cavity. No similar pallial opening was observed in the other species, although the sections were not sufficiently good to positively exclude the presence of such a duct. The thin-walled, ciliated pallial vas deferens runs along the right side of the body wall and is not contained within it. The penis is large, with an expanded distal portion bearing one or more glandular processes (Fig. 8, A–G). There are both epithelial and internal glands. The single, muscular, ciliated penial duct is completely enclosed in the penis and lacks any epithelial connection to the exterior.

Nervous System. This has not been studied in detail but the nervous systems of *Iravadia (Fairbankia) bombayana* and *I. (F.) australis* appear to be generally similar to that of most Hydrobiidae. There is a long supra-oesophageal connective and the sub-oesophageal ganglion lies against the left pleural ganglion.

The Renal System. The kidney is a simple sac with a thin lining except for a cluster of cells on the outer wall.

Key to the genera of the Iravadiidae

1. Shell elongate, sub-cylindrical, translucent, with expanded outer lip ..... *Rissopsis*
   Shell elongate to ovate, opaque to semitranslucent, without expanded outer lip 2
2(1) Shell elongate, smooth or with spiral sculpture, outer lip without varix, sinuate, columella more-or-less vertical ..... *Rhombostoma*
   Shell elongate to ovate, variously sculptured or smooth, outer lip with or without varix, more-or-less straight, columella concave in most species 3
3(2) Operculum with submarginal to marginal nucleus and concentric to sub-spiral growth 4
   Operculum with eccentric nucleus and spiral growth 5
4(3) Shell with spiral sculpture, well-developed in some species, operculum with marginal nucleus ..... *Iravadia*
   Shell with spiral sculpture very weak or absent, operculum with submarginal nucleus ..... *Chevallieria*
5(3) Shell with strongly prosocline outer lip 6
   Shell with approximately orthocline outer lip 8
6(6) Shell with fine spiral threads 7
   Shell smooth or almost so ..... *Hyala*
7(6) Shell with peripheral ridge ..... *Acliceratia* nov.
   Shell lacking peripheral ridge ..... *Ceratia*
8(5) Shell with strong spiral lirae and a prominent varix at outer lip ..... *Liroceratia* nov.
   Shell with weak spiral threads or smooth, without varix at outer lip ..... *Nozeba*

Genus *Iravadia* Blanford, 1867: 56–58

Type-species: *Iravadia ornata* Blanford, 1867 (? = *Pyrgula clathrata* A. Adams, 1853); monotypy. Recent. India.

Synonym: Irawadia, err. auct.

Diagnosis. **Shell.** Elongately ovate-conic to ovate-conic, non-umbilicate to narrowly-umbilicate, with predominantly spiral sculpture, a few species with conspicuous axials or with sculpture reduced to spiral rows of pits. Protoconch planorbid to depressed dome-shaped, of about 2–2½ smooth, convex whorls, usually terminated by a distinct varix. Periostracum often well-developed. **Head-foot.** Cephalic tentacles often banded

or spotted. Snout of moderate length, bilobed. Foot and head pigmented dorsally in most species. Anterior edge of foot usually indented, produced laterally. No pallial tentacles and usually no metapodial tentacle. **Penis**. With broad flattened head and accessory glandular areas. **Oviduct**. With long to short ventral pallial opening; bursa copulatrix present; anterior sperm sac present or possibly absent. **Operculum**. Oval, nucleus on columellar edge, with close, concentric growth lines; some species with weak internal ridge(s). **Radula**. Central teeth wide, each with a usually wide cutting edge bearing one to several, small, sharp cusps; lateral edges unthickened; zero to four basal denticles, weak in most species. Lateral teeth with narrow cutting edge, rather short cusps and long outer portion. Marginal teeth long, curved, with several small cusps.

Distribution and habitat. Indo-Pacific; in brackish-water or in shallow-water, sheltered marine environments to the continental shelf.

Remarks. The genus *Iravadia* possesses a peculiar operculum with a lateral nucleus and concentric growth rings. Although no satisfactory explanation can be offered for the development of this type of operculum, it is assumed that it arose early in the Tertiary, probably from the *Chevallieria* lineage. Evidence for this assumption is the existence of an operculum intermediate in structure in a Recent species attributed to *Chevallieria* and described below. Thiele (1928), on the basis of the structure of the operculum, created two new subfamilies, one for *Fairbankia* in the Micromelaniidae and the other for *Iravadia* in the Hydrobiidae. His placement of these genera in two different families was based on the presence of lateral (basal) denticles on the central teeth of the radula in *Iravadia* and the assumed absence of these in *Fairbankia*. The virtually identical opercular characters and the general similarity of the radula led Brandt (1968, 1974) to include both genera in the Iravadiidae. Because a continuum in shell and radular characters can be observed between typical species of *Iravadia* and *Fairbankia* these two groups are regarded here as being congeneric. There are, however several important differences in the female genitalia of the type-species of these two groups. Examination of an additional species of *Iravadia* s.s., *I. quadrasi,* indicates that considerable differences in the female genitalia may exist within the groupings as here recognised. Similarly, differences between *I. (Fairbankia) australis* and *I. (F.) bombayana* are also marked, although not so radically.

The taxonomy adopted is conservative in that some traditional characters (especially the operculum) are given considerable weight. There are too many gaps in the available information for genital characters to be used in a primary way at the genus-group level at this time, although, undoubtedly they will ultimately be extremely valuable in refining the classification.

Several subgenera are used because these appear to be recognisable groupings, although difficult to define clearly at a higher level. No doubt, when more anatomical information is available, some will be discarded and others may be elevated to generic rank.

The five subgenera recognised show a wide diversity of shell sculpture but are generally similar in other shell features and in their radular and opercular characters as well as in the external appearance of the head-foot. *Iravadia* appears to have diversified from a marine group (subgenus *Pseudonoba*) which has an ancestry traceable at least to the Miocene, and possibly to the Eocene, of Europe. Several species here included in *Iravadia (Pseudonoba)* live in brackish waters or in sheltered, shallow coastal waters; others live on the continental shelf. The incursion into brackish water by members of this genus possibly took place on more than one occasion (Fig. 22).

Key to subgenera of *Iravadia*

1. Shell ovate-conic, with heavy spiral cords (two to six on penultimate whorl); operculum with nucleus in middle of columellar edge 2
   Shell ovate-conic to narrowly-elongate, smooth or with weak to moderate spiral sculpture (more than four cords on penultimate whorl); operculum with nucleus in middle of columellar edge or displaced from middle 3
2(1) Shell with distinct axial ribs; operculum without internal ridges .... *Pseudomerelina* nov.
   Shell with axial ribs or threads; operculum with two internal ridges radiating from nucleus in middle of columellar edge ..... *Iravadia* s.s.

3(1) Shell smooth or with weak to moderate spiral cords and fine axial threads or lamellae 4
Shell with spiral rows of punctures ..... *Fluviocingula*
4(3) Outer lip of aperture evenly prosocline, base of shell without distinct fold ... *Fairbankia*
Outer lip of aperture slightly prosocline, orthocline, or weakly opisthocline, with shallow anterior and posterior channels, base of shell with distinct fold in most species ..... *Pseudonoba*

Subgenus *Iravadia* s.s.

Diagnosis. **Shell**. Of moderate to small size, ovate-conic to elongate-conic, nonumbilicate, with strong spiral cords and weak axial threads to axial ribs. Aperture oval, subangled posteriorly and anteriorly, peristome usually thick; outer lip with heavy varix, slightly prosocline. Protoconch (Figs. 9F, 10B) as for genus. Periostracum well developed. Figs. 9A, F, H; 10A, B. **Head-foot**. Cephalic tentacles with narrow, black bands (in *I. ornata* and *I. quadrasi*) and white spots. Foot with almost straight to indented anterior edge, indented (*I. angulata*), rounded (*I. ornata*) or pointed (*I. quadrasi*) posterior end. No metapodial tentacle. Figs. 1B, E. (*I. ornata*, Hong Kong (New Territories), *I. quadrasi*, Singapore and Darwin, *I. angulata*, Darwin). Figs. 1B, E. **Penis**. (Not examined in *I. angulata*) Bent forwards on itself when at rest, with aperture on inner edge in middle of broad distal half. Outer portion of distal half lamella-like, inner part thick, rugose (*I. ornata*, Fig. 8A) or both sides rugose (*I. quadrasi,* Fig. 8B). **Oviduct**. Known for *I. quadrasi* (Fig. 4) and *I. ornata* (Fig. 21G). Anterior sperm sac present and long oviducal opening in middle of capsule gland in *I. quadrasi*; bursa copulatrix a posterior, ventral sac. Bursa copulatrix on right side and laterodorsal to capsule gland and albumen gland in *I. ornata* and bursal duct opens immediately behind short subterminal opening on left side. A single seminal receptacle in both species. **Operculum**. As for genus, with nucleus midway on columellar edge. Two low radial folds emerge from nucleus and cross about two-thirds of inner surface of operculum (*I. ornata, I. angulata, I. quadrasi*). Figs. 9B, G; 10D, E. **Radula**. Central teeth wide, low, each with short, tongue-like projection in middle of ventral edge and a single, short, weak denticle just inside each unthickened lateral edge (absent in *I. angulata*); a second pair of weak denticles on some central teeth of some specimens of *I. quadrasi*. Lateral and marginal teeth as for genus; lateral teeth with only a long, primary cusp and a small cusp inside this in *I. angulata*; multicuspate in the other two species examined (*I. ornata, I. quadrasi*). Figs. 9C–E; 10C.

Distribution. Southeast Asia as far N as Hong Kong (New Territories) (*Iravadia ornata* Blanford, 1867 ? = *Pyrgula clathrata* A. Adams, 1853, = *Iravadia princeps* Preston, 1915 ? = *I. funera, I.ennurensis* and *I. annandalei* Preston, 1916). Central Indo-Pacific (*Alvania quadrasi* Boettger, 1893 = *Rissoa garretti* Tate, 1899, *nom. nov. pro R. venusta* Garrett, 1873, *non* Philippi, 1844 = *Rissoa (Alvania) alveata* Melvill & Standen, 1901 = *Merelina humera* Laseron, 1956 = *Merelina goliath* Laseron, 1956 = *Merelina reversa* Laseron, 1956 = *Planapexia quadrina* Laseron, 1956 = *Iravadia reticulata* Brandt, 1968). Northern and NE Australia (*Rissoina carpentariensis* Hedley, 1912 = *Pellamora amplexa* Laseron, 1956 = *Pellamora truncata* Laseron, 1956); *Pellamora capitata* Laseron, 1956 = *Pellamora spiralis* Laseron, 1956; *Pellamora angulata* Laseron, 1956).

Habitat. Iravadia ornata was collected under stones in the lower littoral, in an estuarine situation, but not associated with extant mangroves. *Iravadia angulata* and *I. quadrasi* have been found in mangroves under objects in shallow pools. Brandt (1974) records *I. ornata* and *I. quadrasi* (= *reticulata* Brandt) as living in brackish water in the drainages of mud flats, nipa palm and mangrove swamps, and in the estuarine area of rivers. They were found partly buried in mud, feeding on decaying organic material.

Material Examined. I. ornata. Specimens ex Blanford and several other lots (AMS). *R. carpentariensis.* Holotype, paratypes and several other lots (AMS). *Pellamora* species of Laseron, 1956. Types (AMS) and other lots of *P. capitata* and *P. angulata* (AMS). *A. quadrasi.* Lectotype, paralectotypes (SMF) and many other lots (AMS). *P. quadrina.* Holotype (AMS). *M. goliath.* Holotype and paratypes (AMS). *R. garretti.* Holotype (ANSP). *R. (A.) alveata.* Holotype (BMNH). *M. humera.* Holotype AMS). *M. reversa.* Holotype and paratypes (AMS).

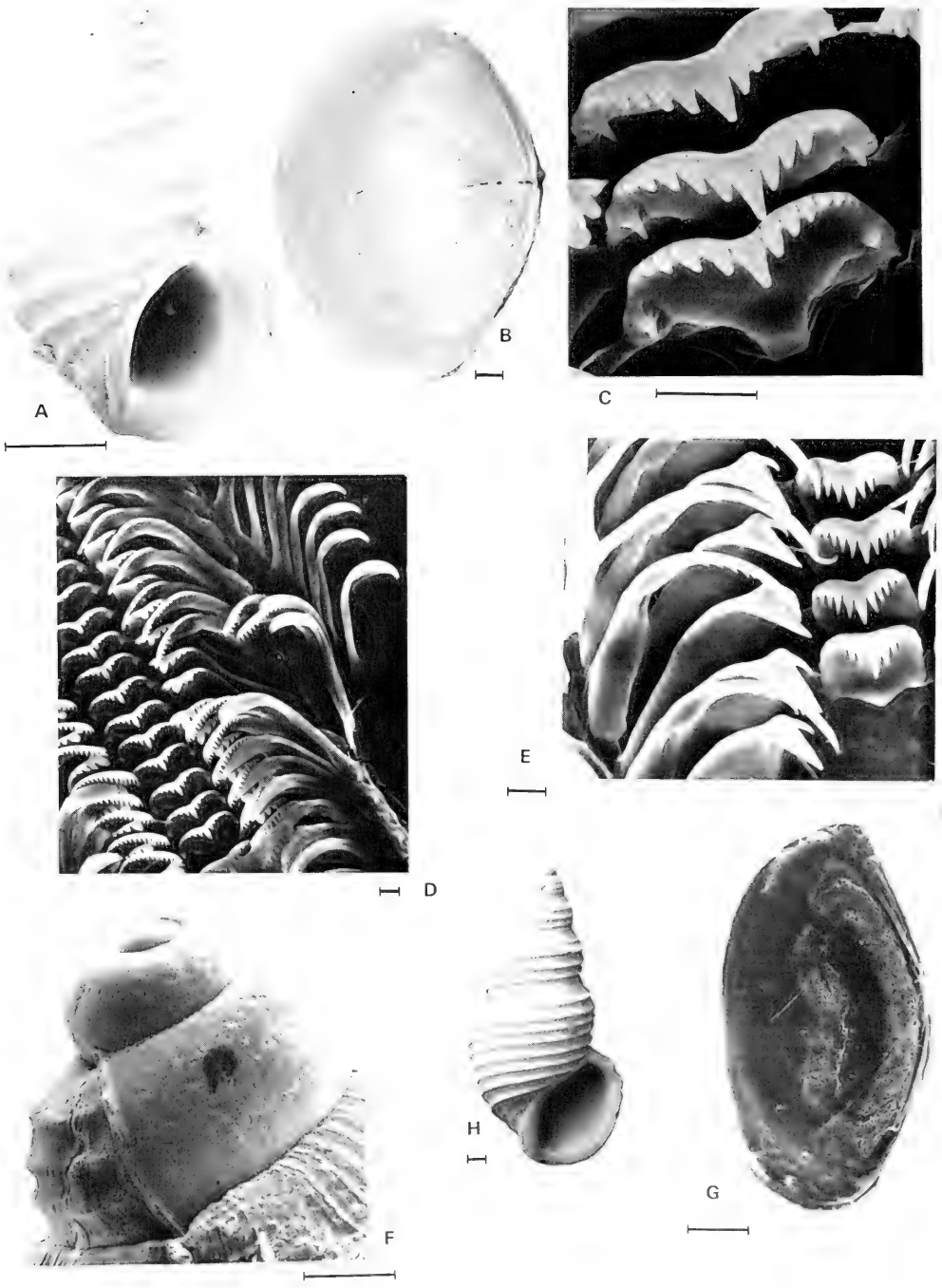

FIG. 9. A–D. *Iravadia (Iravadia) ornata* (Blanford), type-species of *Iravadia*. Deep Bay, New Territories, Hong Kong (AMS). A. Shell. B. Operculum, inner side. C, D. Radula. C. Central teeth only. E–H. *Iravadia (Iravadia) angulata* (Laseron). Norman River, Gulf of Carpentaria, Queensland, Australia (AMS). E. Radula. F. Protoconch. G. Operculum, inner side. H. Shell. Scales: shells = 1 mm; opercula and protoconchs = 0.1 mm; radulae = 0.01 mm.

Remarks. The type-species, *I. angulata* and *I. quadrasi* are estuarine and, whereas the other two Australian species apparently have not been collected alive, they probably have a similar habitat.

Iravadia angulata differs from *I. ornata* in shell characters (compare Figs. 9A and H), the shell being more like that of species of *Iravadia (Fairbankia)* in its tall spire, relatively weak spiral cords, thin peristome and weak varix. The angulated whorls are, however, atypical of the subgenus *Fairbankia*. The operculum (Fig. 9G) is nearly identical to that of *Iravadia ornata* (Fig. 9B) and it is mainly because of the similarity in this structure that *I. angulata* is tentatively included in *Iravadia* s.s. The radula of *I. angulata* has central teeth (Fig. 9E) lacking any basal denticles and the lateral teeth are virtually unicuspid and differ from other species examined in the genus in this respect, except for two new species of *I. (Pseudonoba)* described in the Appendix and discussed below. *Iravadia quadrasi* (Fig. 10A) has more pronounced axial sculpture than *I. ornata* but agrees in other respects.

The differences in the female genitalia of *I. ornata* and *I. quadrasi* are considerable but other species of *Iravadia* should be examined to determine the limits of variation of the female genitalia before further subdivision of this group is proposed.

Subgenus *Pseudomerelina* Ponder, n. subgen.

Type-species: *Alvania mahimensis* Melvill, 1893. Recent, Bombay, India.

Diagnosis. **Shell**. Ovate-conic, with oval aperture, anterior subangulation of aperture absent; sculpture of axial ribs and spiral cords, gemmate at points of intersection. Aperture oval, not markedly subangled posteriorly, rounded anteriorly; outer lip prosocline, varix strong. Protoconch (Fig. 10J) as for genus. Periostracum thin. Figs. 10I, J. **Head-foot**. With conspicuous pigmentation on snout and bands on tentacles. Cephalic tentacles with short 'setae' distally and active cilia in spiral series along rest of tentacles. Foot weakly cleft anteriorly. Posterior end of foot with a weak indentation and a very short, flattened tentacle bearing short, stationary cilia. (Specimens examined from Darwin and Magnetic Island, Australia and Singapore). Fig. 1C. **Penis**. Flattened, with two protuberances, one small, glandular swelling at about half-length on outer side and a flattened section near distal end on outer side. Penial duct in rounded, distal lobe on inner side. Fig. 8C. **Oviduct**. With small anterior bursa copulatrix and pallial, dorsal, posterior bursa copulatrix with narrow, vertical duct. Oviducal opening short, anteriorly placed. Two seminal receptacles (Fig. 5). **Operculum**. As in *Iravadia* s.s. but without internal ridges. An irregular, thickened area inside columella edge in a few specimens. Fig. 10F, G. **Radula**. As in *Iravadia* s.s., with one basal denticle on central tooth. Lateral teeth (1–3) + 1 + (3–4). Fig. 10H.

Distribution. Southeast Asia, India, and central Indo-Pacific to tropical Australia. (*Alvania mahimensis* Melvill, 1893 = *Merelina sucina* Laseron, 1956 = *Merelina solida* Laseron, 1956 = *Iravadia tuberculata* Brandt, 1974).

Habitat. Seaward edge of mangroves, especially on the edge of creeks, on weed, etc., and objects in small pools. Usually abundant when present.

Material Examined. A. mahimensis. Two syntypes (BMNH) and several other lots (AMS). *M. sucina.* Holotype and paratypes (AMS). *M. solida.* Holotype (AMS).

Remarks. This subgenus is proposed for a single species, the shell of which is distinguished from those included in *Iravadia* s.s. by its gemmate sculpture, relatively weak spiral cords, two purple spiral bands on the body whorl, and more evenly-oval aperture. The radula is almost identical to that of *Iravadia ornata* but the operculum, although similar in shape, lacks any internal ridges. *Iravadia (Pseudomerelina) mahimensis* is the only species of the Iravadiidae known to possess a metapodial tentacle (Fig. 1C). It has presumably disappeared in the other species of the family examined alive, although there is a low ridge on the posterior end of the foot in *I. quadrasi* (Fig. 1B). In addition, the penis is of simpler construction than in the other subgenera of *Iravadia*; it has a less swollen distal portion and only two small accessory protuberances; and there are two seminal receptacles in the female genital system, not one as in other members of the genus.

Iravadia (Pseudomerelina) mahimensis lives in an estuarine habitat and in the sheltered waters of enclosed bays and appears to have a wide distribution through the central Indo-Pacific.

The shells of *Iravadia (Pseudomerelina)*

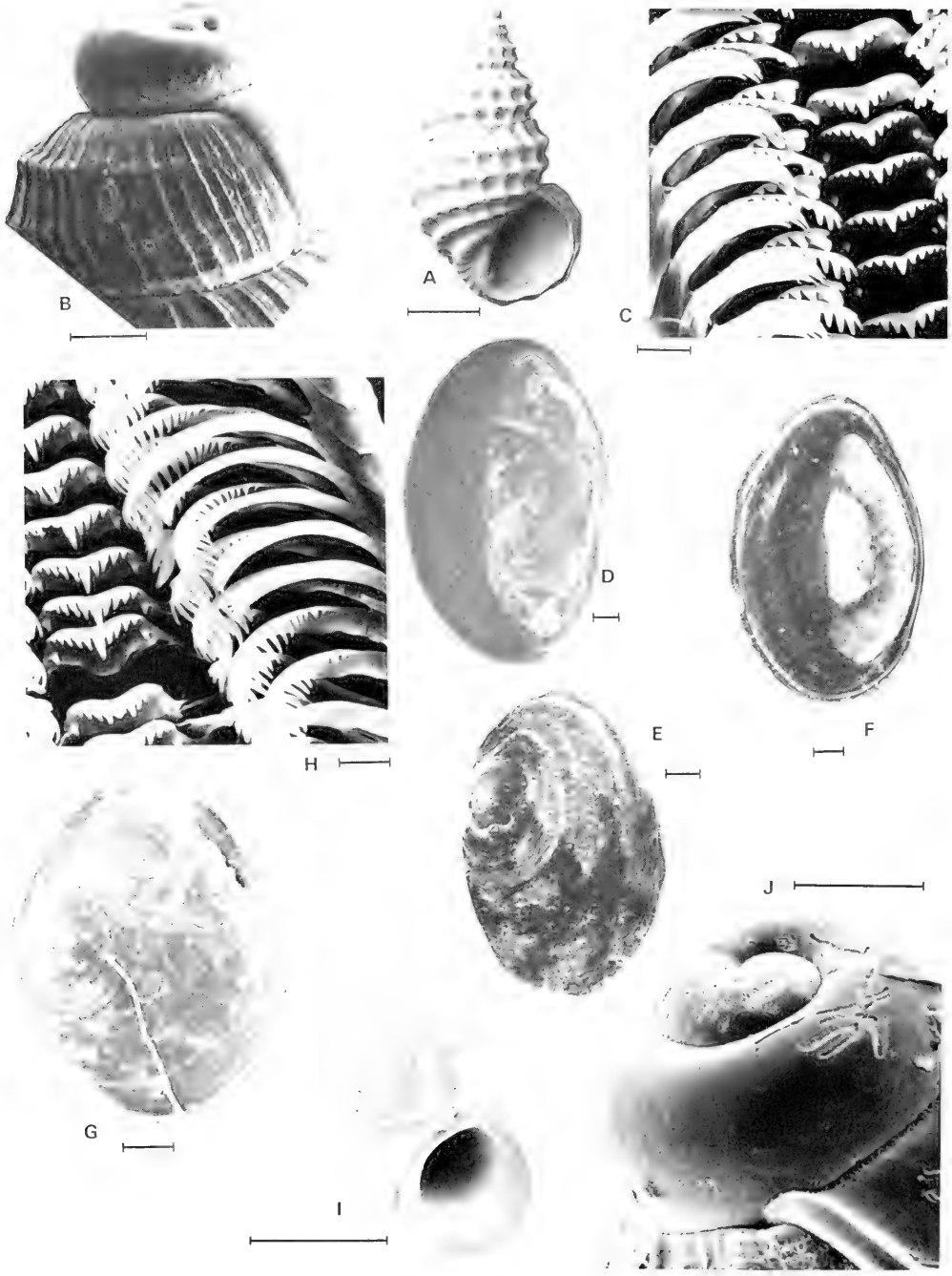

FIG. 10. A–E. *Iravadia (Iravadia) quadrasi* (Boettger). A. Shell. B. Protoconch. C. Radula. D–E. Operculum, inner (D) and outer (E) sides. A, B, D. Proserpine River estuary, Wilson, Queensland, Australia. C. Gatakers Bay, Hervey Bay, Queensland. E. Mouth of Brisbane River, Queensland. All AMS. F–J. *Iravadia (Pseudomerelina) mahimensis* (Melvill). Type-species of *Pseudomerelina* nov. F, G. Operculum, inner (F) and outer (G) sides. H. Radula. I. Shell. J. Protoconch. F. Magnetic Island, Queensland, Australia. G–J. Maningrida, Arnhem Land, Northern Territory, Australia. Both AMS. Scales: shells = 1 mm; opercula and protoconchs = 0.1 mm; radulae = 0.01 mm.

mahimensis (Fig. 10I) and *Iravadia (Iravadia) quadrasi* (Fig. 10A) are superficially very similar to species of *Alvania* and *Merelina* (Rissoidae) but can be distinguished by their prosocline outer lips and their small, smooth, flattened protoconchs.

Subgenus *Fairbankia* (Blanford MS) Stoliczka, 1868 (July): 274

Type-species: *Fairbankia bombayana* (Blanford MS) Stoliczka, 1868, original designation. Recent, Bombay, India.

Synonyms: Pellamora Iredale, 1943: 206. Type-species: *Iravadia australis* Hedley, 1900; original designation. Recent, NE Australia.

Wakauraia Kuroda & Habe, 1954: 75. Type-species: *Fairbankia (Wakauraia) sakaguchii* Kuroda & Habe, 1954. Recent, Japan.

Diagnosis. **Shell**. Elongately-conic, non-umbilicate, solid, with weak to moderate spiral sculpture and weak axial threads or lamellae. Aperture relatively small, weakly to distinctly angled anteriorly, very weakly channelled posteriorly, outer lip slightly to moderately prosocline. Protoconch as for genus. Periostracum moderately well-developed, with short processes in some species. Fig. 11A, F. **Head-foot**. Cephalic tentacles very slightly 'setose' distally, with or without a few darkly pigmented bands or black spots. Head and foot pigmented dorsally. Anterior edge of foot indented, posterior end blunt, with shallow indentation. No metapodial tentacle. (*I. (F.) australis, I. (F.) bombayana*). Fig. 1A, D. *I. (F.) cochinchinensis* and *I. (F.) rohdei* appear to be similar according to Brandt's (1974) description. **Penis**. (*I. (F.) australis*). Short, compressed, with a row of accessory glandular swellings below an expanded distal portion on which penial duct opens subterminally. Fig. 8D., *I. (F.) bombayana* with broad, simple head; only one glandular area apparent in middle region (Fig. 8G). **Oviduct**. Anterior sperm sac absent (*I. (F.) bombayana*) or very reduced (*I. (F.) australis);* bursa copulatrix with vertical opening separated from oviducal opening. Oviducal opening short and anteriorly placed (*I. (F.) australis*) or occupying anterior two-thirds of capsule gland (*I. (F.) bombayana*). A single seminal receptacle present. Figs. 6, 7. **Operculum**. Oval, with nucleus approximately two thirds along columellar edge, growth-lines concentric, a more-or-less longitudinal, low, internal ridge present (*I. (F.) bombayana* and *I. (F.) australis*). *I. (F.) sakaguchii* is similar (Kuroda & Habe, 1954, text fig. 12). Fig. 11D, E, G. H. **Radula**. Similar to that of *Iravadia* s.s. but central teeth with two or three weak, basal denticles placed against upper lateral margins and just beneath outer, dorsal cutting edge (*I. (F.) bombayana* and *I. (F.) australis*). *I. (F.) sakaguchii* is similar but no basal denticles are shown in the illustration of the central tooth (Kuroda & Habe, 1954, text fig. 11). Fig. 11B, C, I, J.

Distribution. South and SE Asia, Sumatra, S China, Philippines (*F. bombayana* = *Fairbankia quadrasi* Boettger, 1893; *Fairbankia cochinchinensis* Bavay & Dautzenberg, 1940; *Fairbankia rohdei* Brandt, 1968; *Onoba tenuilirata* Boettger, 1893). Northern and NE Australia (*Iravadia australis*). Red Sea (?*Onoba elongata* Hornung & Mermod, 1928). Japan (*F. (W.) sakaguchii*).

Habitat. Mangroves, under objects in shallow pools (*I. (F.) australis*) or on the surface of mud in standing water (*I. (F.) bombayana*). Drainage system of mudflats, nipa palm and mangrove swamps (*I. (F.) bombayana, I. (F.) rohdei*, Brandt, 1974).

Material Examined. F. bombayana. A few lots (AMS, BMNH). *F. quadrasi*. One lot (AMS). *F. cochinchinensis*. Three lots, ex Brandt (USNM). *I. australis*. Holotype, paratypes and several other lots (AMS). *F. rohdei*. Four lots (USNM). *O. tenuilirata*. Holotype (SMF). *O. elongata*. Holotype (GNHM). *F. (W.) sakaguchii*. Paratypes (USNM).

Remarks. The species in this subgenus differ from those in *Iravadia* s.s. in their more elongate shells with weaker spiral sculpture, more oval apertures, and the bursa copulatrix has a vertical opening separate from that of the capsule gland. The central teeth of the radula have only weak, dorso-laterally placed basal denticles and the nucleus of the operculum is displaced from the middle of the columellar edge. The species of *Iravadia (Fairbankia)* live in an estuarine habitat, usually amongst mangroves, like those of *Iravadia* s.s.

Brandt (1968) described the radula, operculum and animal of his new species *F. rohdei* from Thailand and Thiele (1928) described the radula and operculum of *F. bombayana*. These species have radulae and opercula very similar to those of *Iravadia ornata*; Blanford (1868) and Brandt (1968, 1974) recognised them as closely related genera. Brandt (1974) pointed out the similar-

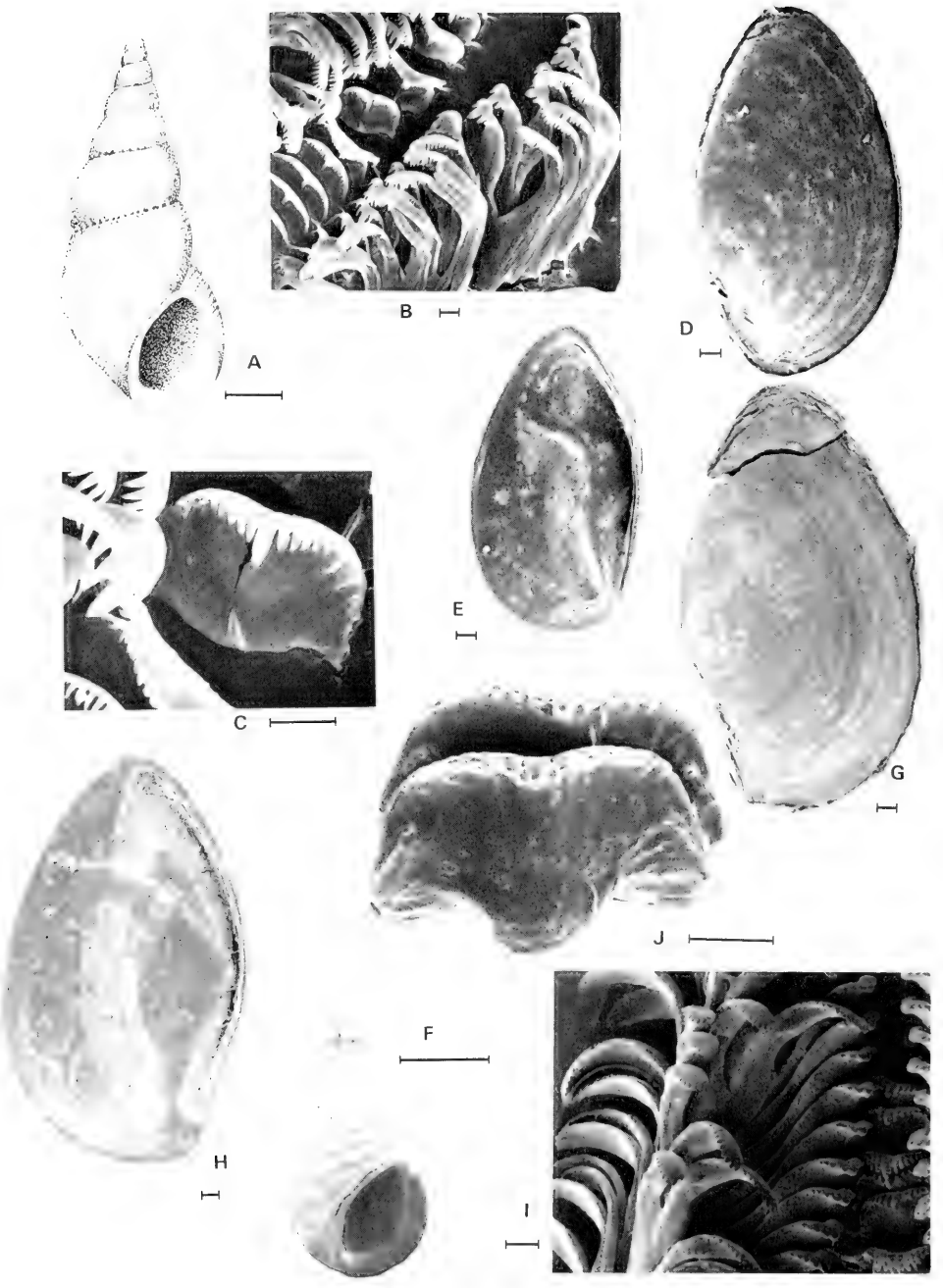

FIG. 11. A–E. *Iravadia (Fairbankia) bombayana* (Stoliczka), type-species of *Fairbankia*. A. Shell. B, C. Radula, central tooth. D–E. Operculum, outer (D) and inner (E) sides. A, D, E. Bombay, India (ex Blanford) (BMNH). B, C. Sembawang Estuary, Singapore (AMS). F–I. *Iravadia (Fairbankia) australis* (Hedley), type-species of *Pellamora*. F. Shell. Paratype, Bowen, Queensland, Australia (AMS). G, H. Operculum, outer (G) and inner (H) sides. I, J. Radula. G–J. Magnetic Island, Queensland, Australia (AMS). Scales: shells = 1 mm; opercula = 0.1 mm; radulae = 0.01 mm.

ity of the shells of *Fairbankia bombayana* to *Mainwaringia* Nevill, 1884 (type-species *Melania (Mainwaringia) paludomoidea* Nevill, 1884) but the radula as figured by Annandale & Prashad (1919) is dissimilar and the operculum is described as "horny, extremely thin, paucispiral, with the nucleus eccentric." Certainly the characters of the radula and operculum remove *Mainwaringia* from any close association with *Fairbankia.*

The shells of the type-species of *Pellamora* and *Fairbankia* differ in the relative strength of the spiral sculpture. A gradation is seen, however, in three SE Asian species, from weak spiral sculpture in *I. (F.) bombayana* to distinct spirals in *I. (F.) cochinchinensis* to moderately strong spirals in *I. (F.) rohdei.* The spiral sculpture of this last species approaches that of *I. (F.) australis* (Fig. 11F). *Iravadia angulata* is, on the other hand, very similar in shell characters (Fig. 9H) to *I. (F.) australis.* The type-species of *Wakauraia* is, as admitted by its authors, very similar to other species of *Fairbankia.* The main difference appears to be in the degree of angulation of the anterior end of the aperture. As this character alone is not considered to be of subgeneric importance, *Wakauraia* is regarded here as a synonym of *Fairbankia.*

Blanford (1868) published the description of his new genus (*Fairbankia*) in December, 1868. In his discussion he cites the Stoliczka (1868) reference in which his genus name was inadvertently introduced in July, 1868. Because Stoliczka gives a full description of the genus and species, he should be regarded as the author of *Fairbankia.*

Subgenus *Fluviocingula* Kuroda & Habe, 1954: 73

Type-species: *Fluviocingula nipponica* Kuroda & Habe, 1954; original designation. Recent, Japan.

Synonym: Mesodestea Laseron, 1956: 451. Type-species: *Mesodestea resima* Laseron, 1956; original designation. Recent, northern Australia.

Diagnosis. **Shell**. Small, ovate-conic, rather thin, usually narrowly-umbilicate with evenly-convex whorls, sculptured with very weak, scarcely-raised spiral cords and axial threads, interspaces forming shallow pits (Fig. 12D). Aperture oval, with very weak anterior and posterior angulations, peristome thin, varix weak, outer lip prosocline. Protoconch as for genus. Periostracum rather thin. Fig. 12A, B, D. **Head-foot**. Head pigmented, cephalic tentacles with a broad band of pigmentation and stationary 'setae' distally. Foot cleft anteriorly and posteriorly. No metapodial tentacle (*F. resima*, Darwin, N Australia). Fig. 1F. **Penis** and **oviduct** not known. **Operculum.** Similar to that of species of *Iravadia (Iravadia)* but without internal ridges (*I. (F.) nipponica, I. (F.) resima*). Fig. 12C, H. **Radula**. Central teeth similar to those in *Iravadia* s.s. and *I. (Fairbankia)*, with central teeth showing from none to two laterally placed basal denticles on each side (none or one in *I. (F.) resima*, two in *I. (F.) nipponica*). Fig. 12E–G.

Distribution. Inland Sea of Japan and Sea of Japan (*F. nipponica*). Northern Australia (*M. resima*).

Habitat. Associated with mangroves but at their inshore edge in damp areas (*I. (F.) resima*). Saltwater lagoons and estuaries on mud and *Zostera,* up to 1 m in depth from negative temperatures to 33°C (summer); salinity 4–7‰ (*I. (F.) nipponica*, Golikov & Kussakin, 1978).

Material Examined. F. nipponica. One lot ex Golikov (AMS); paratypes (USNM). *M. resima.* Holotype and a few other lots (AMS).

Remarks. The type-species of *Fluviocingula* and *Mesodestea* are very similar, although the Australian species has a more elongate shell (Fig. 12A, B) and a slightly different radula (compare Figs. 12E, F with G). This subgenus differs from the others included in *Iravadia* by its curious shell sculpture, a series of minute spiral pits (Fig. 12D) between the weak spiral cords. The narrow umbilicus also sets the shell of this group of species apart from the remainder of the genus, although in an undescribed Australian species the umbilicus is absent. The operculum resembles that of species of *Iravadia (Pseudomerelina)*, in lacking any internal ridges.

Golikov & Kussakin (1978) report probable viviparity in *I. (F.) nipponica.*

Subgenus *Pseudonoba* Boettger, 1902: 145

Type-species: *Pseudonoba peculiaris* Boettger, 1902; original designation. Middle Miocene, Rumania.

Synonyms: Sinusicola Kuroda & Habe, 1950 (Jan. 15): 16. Type-species: *Turbonilla (Careliopsis) filiola* Yokoyama, 1927 (= *Rissoina yendoi* Yokoyama, 1927), original designation. Upper Pleistocene, Koyasu, Japan.

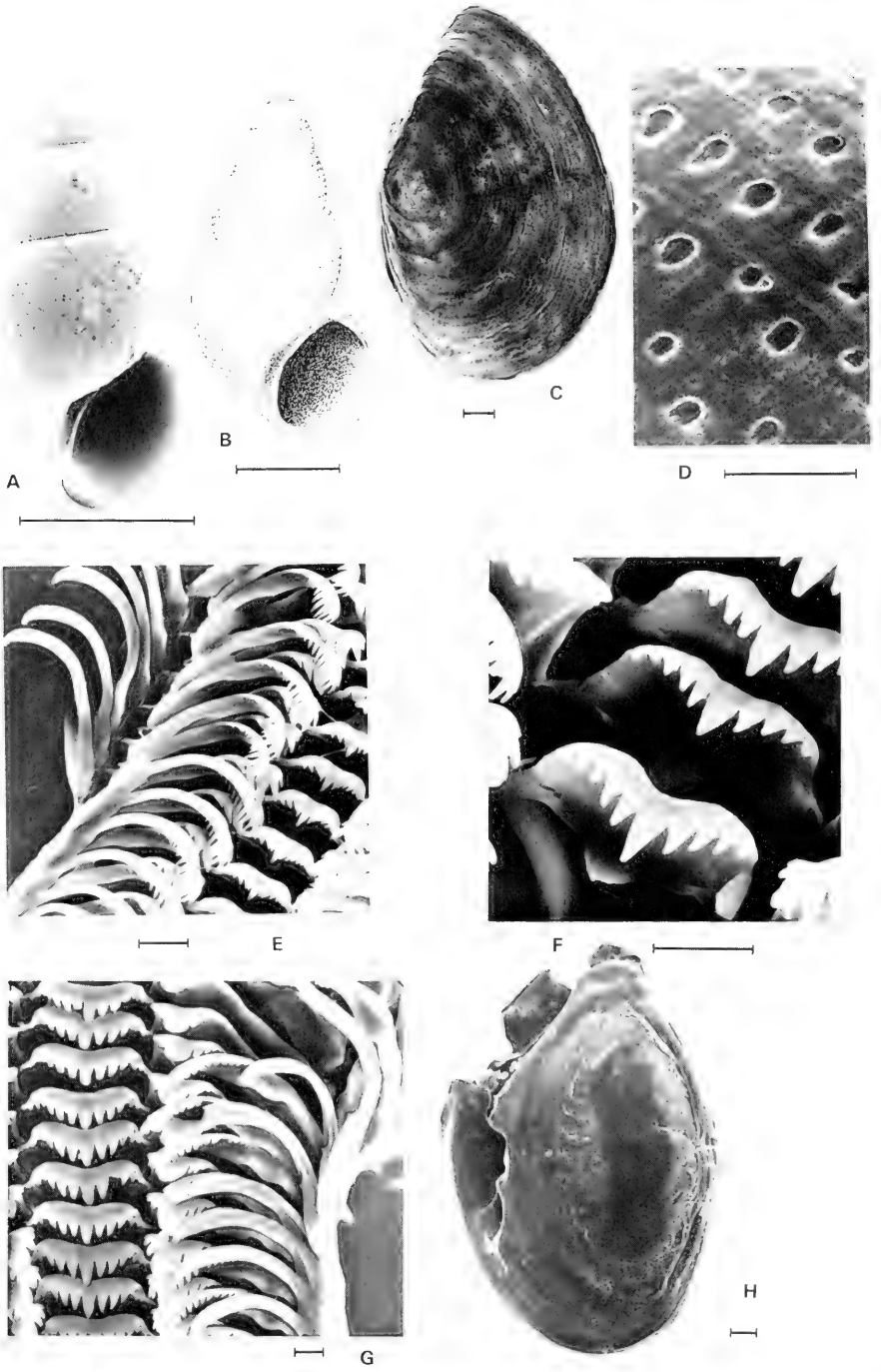

FIG. 12. A–F. *Iravadia (Fluviocingula) resima* (Laseron), type-species of *Mesodestea*. A, B, D: Shell; B, holotype (AMS), D, microsculpture of teleoconch. C. Operculum, outer side. E–F. Radula; F, central teeth. A, C–F. Diana Beach, Darwin, Northern Territory, Australia (AMS). G, H. *Iravadia (Fluviocingula) nipponica* (Kuroda & Habe), type-species of *Fluviocingula*; Posyet Bay, U.S.S.R. (AMS) G. Radula, H. Operculum, inner side. Scales: shells = 1 mm except Fig. D scale = 0.1 mm; opercula = 0.1 mm; radulae = 0.01 mm.

Paronoba Laseron, 1950 (Jan. 27): 283. Type-species: *Paronoba subquadrata* Laseron, 1950; original designation. Recent, SE Australia.

Dipsotoma Laseron, 1956: 416. Type-species: *Rissoa mercurialis* Watson, 1886 (= *Rissoa bella* A. Adams, 1851, ? = *Onoba delicata* Philippi, 1849); original designation. Recent, tropical Indo-Pacific.

Lucidinella Laseron, 1956: 427. Type-species: *Lucidinella conicera* Laseron, 1956 (= *Rissoa (Amphithalamus) densilabrum* Melvill, 1912); original designation. Recent, tropical Indo-Pacific.

Iraqirissoa Dance & Eames, 1966: 39. Type-species: *Rissoa (Amphithalamus) aristaei* Melvill, 1912; original designation. Recent, India.

Diagnosis. **Shell**. Elongately-ovate to narrowly-elongate, nonumbilicate or with shallow umbilical chink; most species with distinct basal fold; sculptured with weak to moderate spirals and weak axial threads. Aperture not much expanded, with thick to moderate varix; outer lip slightly to moderately prosocline, orthocline or weakly opisthocline, usually with distinct but shallow anterior excavation and posterior subangulation. Protoconch as for genus. Periostracum thin, often covered with a reddish brown coating. Figs. 13A–D, I, J; 14A, B, G; 15A, B, E, H; 16A, B, G. **Head-foot**. Cephalic tentacles unpigmented, with spiral bands of cilia. Posterior end of foot slightly indented, anterior end indented. No metapodial tentacle. (*I. (P.) bella,* Magnetic Island, Queensland; *I. (P.)* cf. *aristaei,* Singapore; *I. (P.)* sp., cf. *bella,* Singapore). Fig. 1H. **Penis** and **oviduct** not known. **Operculum**. Elongately-oval, usually with columellar margin indented towards lower end, nucleus in middle of columellar margin or displaced slightly. A weak, longitudinal internal ridge sometimes present (several species examined). Figs. 14C, D, H; 15C, G; 16C, H, I. **Radula**. Similar to other members of the genus except central teeth with more pronounced lateral extensions in some species; one to four small to very large denticles on lateral edges of central teeth. Cutting edge of central teeth broad with numerous denticles, to very narrow, with only three. Lateral teeth with one to several cusps (several species examined—see Remarks). Figs. 14E, F, I; 15D, F; 16D, E, F.

Distribution. Tropical Indo-Pacific (*R. (A.) aristaei, Rissoa (Scrobs) ictriella* Melvill, 1910, = *Rissoa (Amphithalamus) alphesiboei* Melvill, 1912; *Aclis atemeles* Melvill, 1896; ? *Onoba delicata* Philippi, 1849 ? = *Rissoa bella* A. Adams, 1851 = *Rissoa vitrea* Garrett, 1873 = *Rissoa (Onoba) mercurialis* Watson, 1886 = *Onoba philippinica* Boettger, 1893 = *Rissoina oscitans* Preston, 1905 = *Amphithalamus psomus* Melvill, 1918; *Chevallieria padangensis* Thiele, 1925; *Rissoa (Amphithalamus) densilabrum* Melvill, 1912 = *Onoba quadrasi* Boettger, 1893 (secondary homonym of *Iravadia quadrasi* (Boettger, 1893)) = *Lucidinella conicera* Laseron, 1956 = *Lucidinella conicera patruelis* Laseron, 1956; *Lucidinella sublaevis* Laseron, 1956). Late Miocene, western Pacific (Eniwetok Atoll) (*Cingula (Peringiella) parryensis* Ladd, 1966). Southeastern Australia (*P. subquadrata*). Japan (*R. yendoi* = *T. (C.) filiola* Yokoyama, 1927). New Zealand (*Dipsotoma inflata* Ponder, 1968). Middle Miocene, Rumania (*P. peculiaris*). Eocene, France (? *Ceratia (?) allixi* Cossmann, 1922).

Habitat. Marine to estuarine. *I. (P.) bella* and *I. (P.)* sp. cf. *aristaei* were found living in mangroves under objects on the mud or in shallow pools. *Iravadia (P.) padangensis*, and the three new species described in the Appendix are fully marine and live in relatively deep water. *Iravadia (P.) densilabrum* was collected alive under coral blocks at low tide in a fully marine situation.

Material Examined. P. peculiaris. Lectotype and many paralectotypes (SMF), one lot, ex Cossmann (NHMP). *C. (P.) parryensis.* Holotype (USNM). *O. delicata.* One specimen so named (BMNH). *O. bella.* Two probable syntypes ex Adams (NMW) and several other lots (AMS). *R. vitrea.* Five syntypes (ANSP). *R. (O.) mercurialis.* Holotype (BMNH). *O. philippinica.* Lectotype and paralectotype (SMF). *R. oscitans.* Three syntypes (BMNH); two "paratypes" (ANSP). *A. psomus.* Holotype (BMNH). *P. subquadrata.* Six syntypes and several other lots (AMS). *C. padangensis.* Holotype and 11 paratypes (HUM). *O. quadrasi.* Lectotype and one other specimen (SMF). *R. (A.) densilabrum.* Two syntypes (BMNH) and several other lots (AMS). *L. conicera* and *L. conicera patruelis.* Holotypes and paratypes (AMS). *L. sublevis.* Holotype (AMS). *D. inflata.* Holotype (AIM). *T. (C.) filiola.* Holotype (GIT). *R. yendoi.* Holotype (GIT) and one other lot (NSMT). *R. (A.) aristaei.* Lectotype and two paralectotypes (BMNH). *C. (?) allixi.* One lot (topotypes), ex Le Renard.

Remarks. At least some of the species of

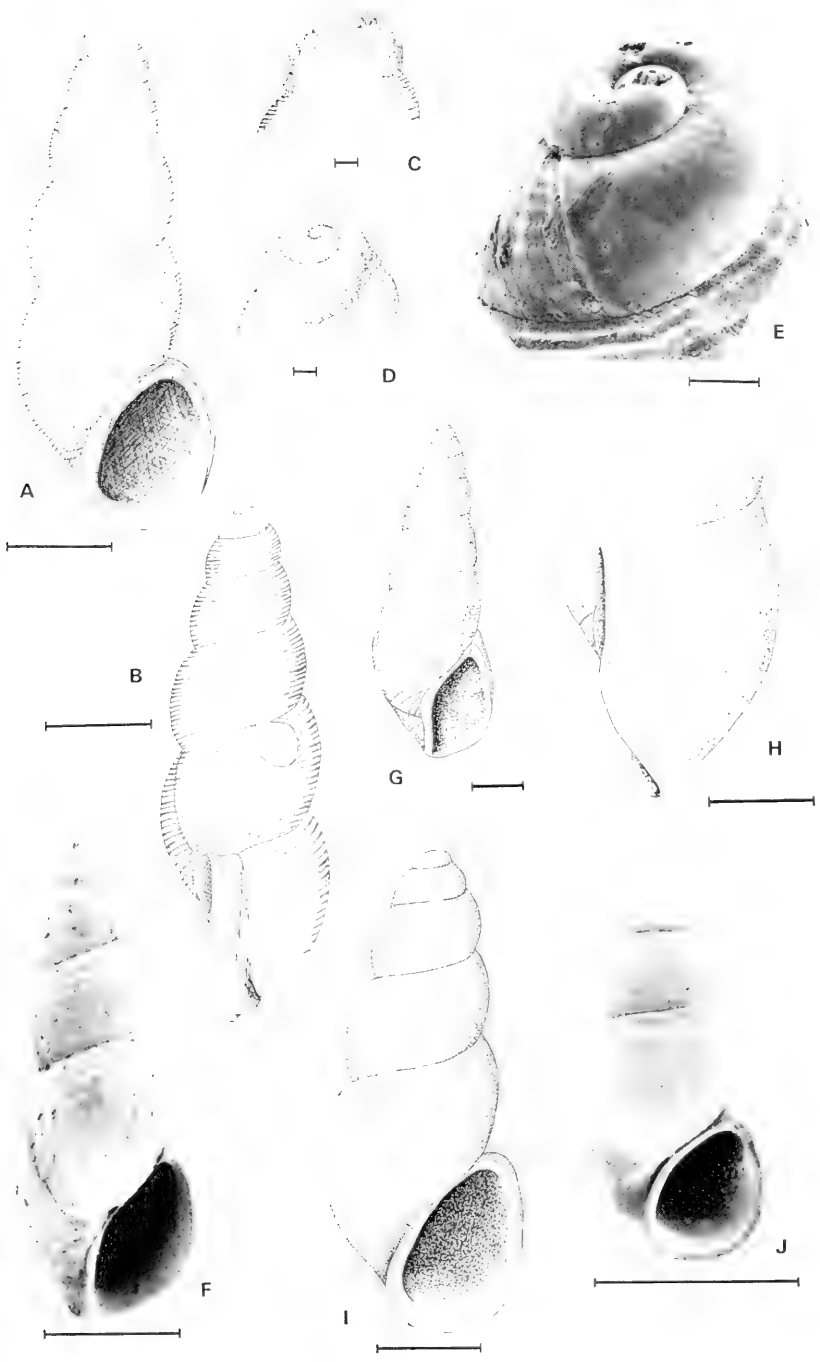

FIG. 13. A–D. *Iravadia (Pseudonoba) peculiaris* (Boettger), type-species of *Pseudonoba*; topotype, Kostej, Rumania (ex Cossmann Colln.) (NHMP). A, B. Shell, front and side views. C, D. Protoconch. E–F. *Rhombostoma imperforatum* (Sacco). Ozciano, Italy, (ZMR). E. Protoconch, F. Shell. G, H. *Rhombostoma carmelae* (Brugnone), syntype (ZMR); type-species of *Rhombostoma*; G, H. Shell. H, side view of aperture. I, J. *Iravadia (Pseudonoba) subquadrata* (Laseron), type-species of *Paronoba*. I, J. Shells; I. holotype. Port Stephens, New South Wales, Australia (AMS). J. Port Hacking, New South Wales (AMS). Scales: shells = 1 mm; protoconchs = 0.1 mm.

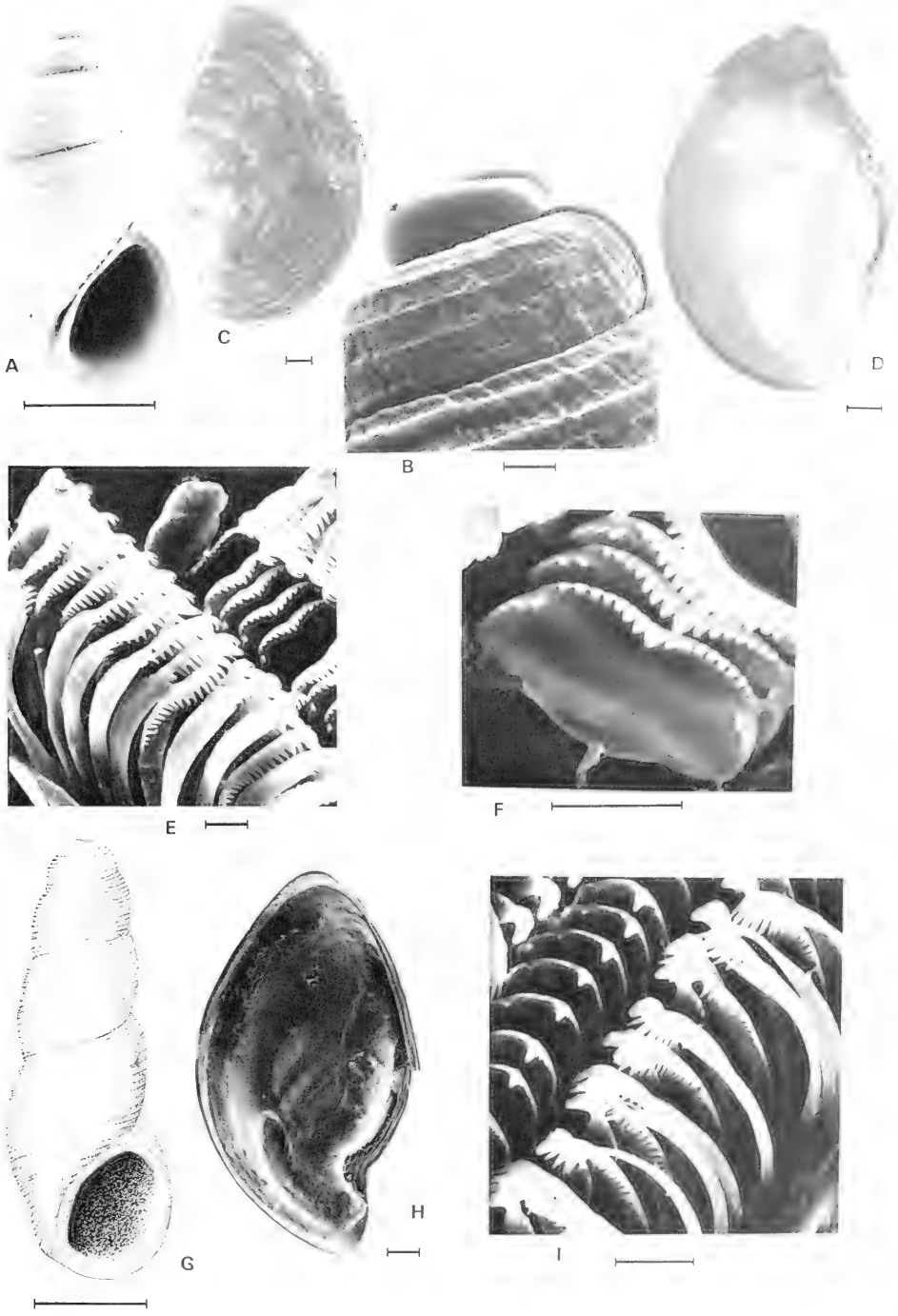

FIG. 14. A–F. *Iravadia (Pseudonoba) densilabrum* (Melvill), type-species of *Lucidinella*. A. Shell. Paratype of *Lucidinella conicera* Laseron, Whitehaven Beach, near Bowen, Queensland, Australia (AMS). B. Protoconch. C, D. Operculum, outer (C) and inner (D) sides. E, F. Radula, F, central teeth. B, C, E. Lindeman Is., Queensland (AMS); D, E. Norsup, Malekula, New Hebrides (AMS). G–I. *Iravadia (Pseudonoba) profundior* sp. nov. G. Shell of holotype. H. Operculum (inner side). I. Radula. H and I from paratype. Scales: shells 1 mm; opercula and protoconchs = 0.1 mm; radulae = 0.01 mm.

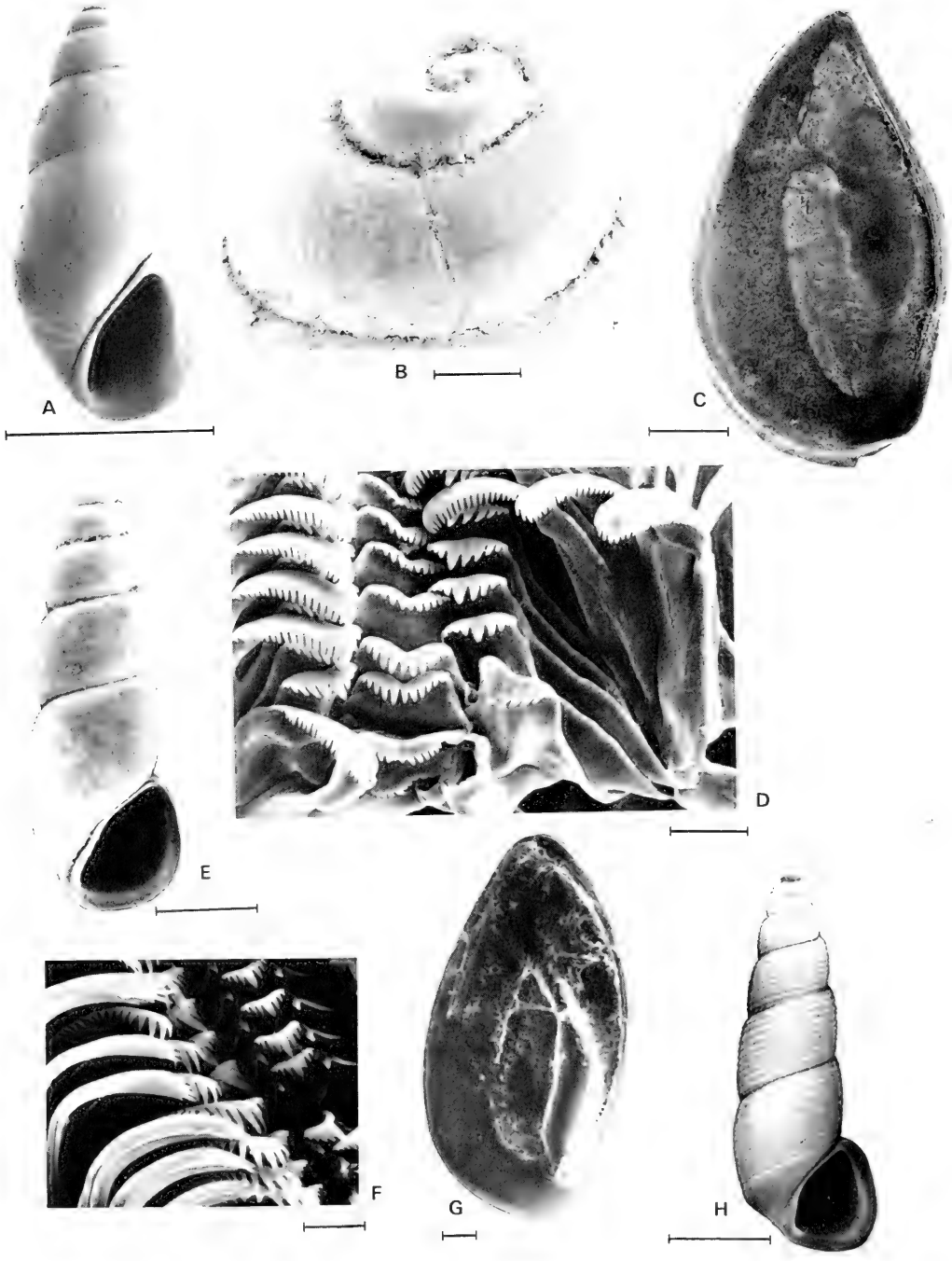

FIG. 15. A–D. *Iravadia (Pseudonoba) bella* (Adams), type-species of *Dipsotoma* Laseron. Magnetic Island, Queensland, Australia (AMS). A. Shell. B. Protoconch, C. Operculum, inner side. D. Radula. E. *Iravadia (Pseudonoba) filiola* (Yokoyama), type-species of *Sinusicola*, Tomioka, Amakusa, Kyushu, Japan (NSMT). Shell. F–H. *Iravadia (Pseudonoba)* sp. Sembawang, Singapore (AMS). F. Radula. G. Operculum, inner side. H. Shell. Scales: shells = 1 mm; opercula and protoconchs = 0.1 mm; radulae = 0.01 mm.

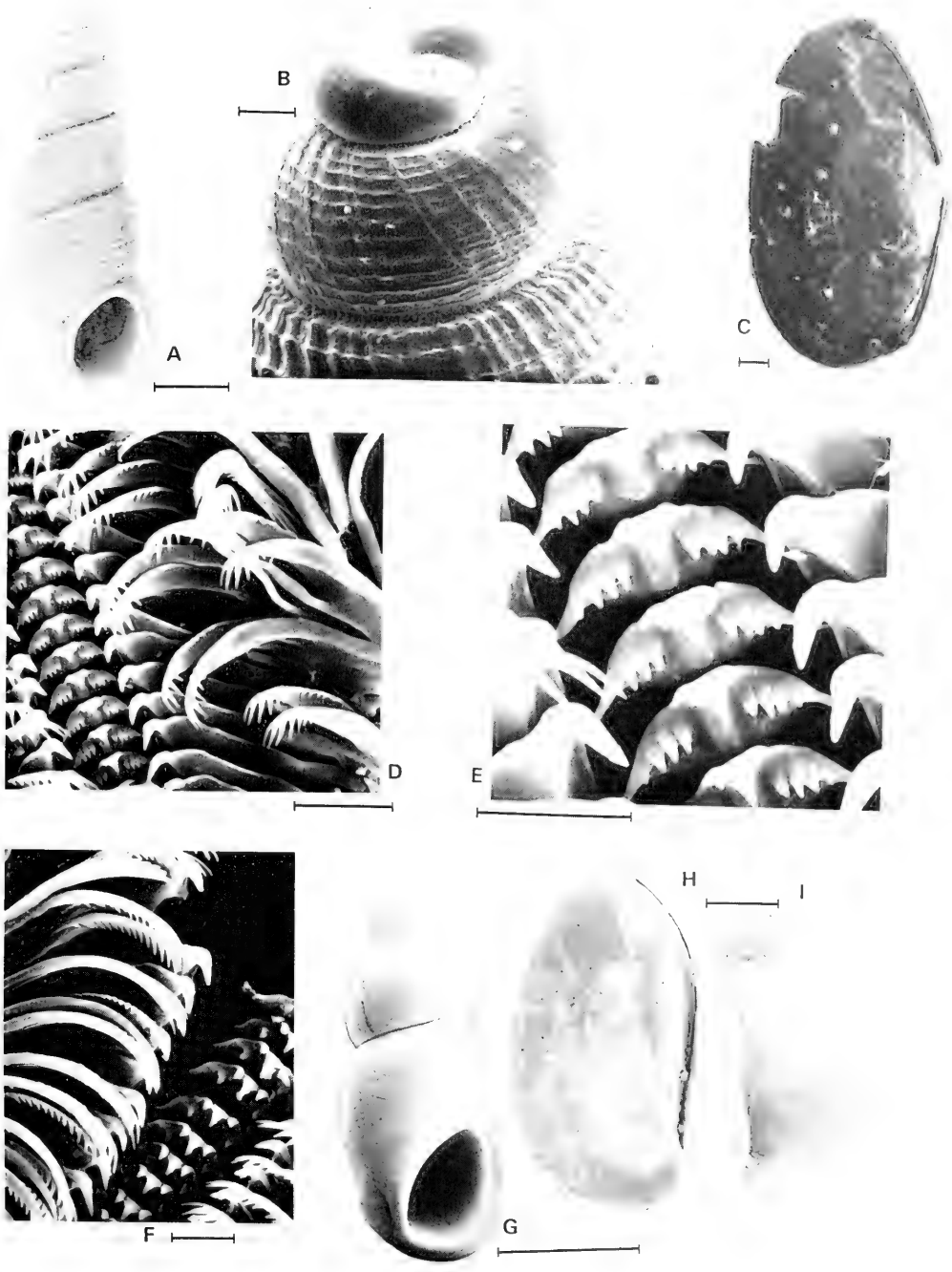

FIG. 16. A–E. *Iravadia (Pseudonoba) gemmata* sp. nov. A. Shell of holotype. B. Protoconch. C. Operculum, D, E. Radula; E, central teeth. B–E, from paratypes. F–I. *Iravadia (Pseudonoba) expansilabrum* sp. nov. F. Radula. G. Shell of holotype. H, I. Operculum, inner (H) and outer (I) sides. F, H, I. from paratype. Scales: shells = 1 mm; opercula and protoconchs = 0.1 mm; radulae = 0.01 mm.

this subgenus are fully marine and range from shallow coastal waters to relatively deep water. The type-species of *Dipsotoma, Sinusicola* and *Iraqirissoa* probably normally live in estuarine conditions.

The shells of species of this subgenus encompass a considerable range of form. There appears to be a gradation from very elongate shells (as in the type-species of *Iraqirissoa* and *Sinusicola* (Fig. 15E)) to others with moderate spires, the latter usually having much less-impressed sutures and flatter whorls than the former. The spiral ornament usually consists of rather weak spiral threads but some species are sculptured with close spiral lirae. Species included in *Lucidinella* by Laseron (1956) have shells with prominent axial threads and a rather strong basal fold. The lower part of the inner lip is separated from this basal fold and an umbilical chink is formed between them. This style of shell is very similar to a relatively weakly and spirally sculptured species of *Iravadia* s.s., *I. capitata* (Laseron), and to some species of *Iravadia (Fairbankia). Paronoba subquadrata* (Figs. 13I, J) is somewhat intermediate in the basal features of the shell in sometimes having a basal fold and thus forms a link between *Lucidinella conicera (= densilabrum)* (Fig. 14A), which always has a basal fold and *Dipsotoma mercurialis (= bella)* (Fig. 15A), which lacks one. This last species resembles the type-species of *Pseudonoba* (Fig. 13A, B) in all essential shell features.

Habe (1958) described the radula and operculum of *Sinusicola endoi (sic!, = yendoi),* which agree closely with those of the type-species of *Fairbankia, Pseudomerelina* and species of *Lucidinella,* although the structure of the inner side of the operculum was not noted. The shell of this species (Fig. 15E) is smaller than most of the type-species included in the synonymy of *Pseudonoba* but agrees generally with them in shape and sculpture. It is also similar to species in the subgenus *Fairbankia* in shape but is smaller in size and has an orthocline, not prosocline, outer lip. A very similar species, *Rissoa aristaei* Melvill, from Bombay, is the type-species of *Iraqirissoa.* Specimens from Singapore of a possibly undescribed species similar to both *R. aristaei* and *T. filiola* (Fig. 15H) differ in the characters of the central teeth of the radula from the figure of the radula of *S. yendoi (= T. filiola)* (Habe, 1958), but have an operculum like that of species of *Fairbankia* and *Pseudonoba* (Fig. 15G). These species appear to fall between *Fairbankia* and *Dipsotoma* in shell features but are here included somewhat tentatively in *Pseudonoba.* The common shell features outlined in the generic diagnosis are a combination of characters which tie the type-species of the genera listed in synonymy together. Further support for their close relationship is gained by the very similar opercular characters seen in the species examined, which encompass the majority of the observed shell variations within the subgenus.

Unfortunately, the radular and opercular characters of some of the type-species of the genus-group names included in the synonymy are not known, so this grouping must be considered somewhat tentative. The type-species of *Pseudonoba* is a Miocene fossil but the radula and operculum of a closely similar new species (see Appendix) have been examined (Fig. 14G–I). *Paronoba subquadrata* is also known only from its shell which is, as noted above, similar to *P. peculiaris* and also to *Dipsotoma mercurialis* in all essential features. The shell of the type-species of *Iraqirissoa* is similar but more elongate than the shells of the type-species of *Pseudonoba* and *Dipsotoma.* It also has a rather weak basal fold and weak spiral sculpture.

Pseudonoba is probably more closely related to *Fairbankia* than to *Iravadia*, as far as can be judged from the shell, radula and operculum. The probable greater antiquity and mainly marine habitat of species of *Pseudonoba* suggest that it is the ancestral group within the genus *Iravadia.*

There is very considerable variation in the radular teeth, particularly in the central teeth, in the species examined in this group. This may indicate that the group is polyphyletic but it is also possible that because it is of some antiquity and considerable geographic spread this is divergence within a single phyletic group. The 'normal' *Iravadia* radula is seen in some species (Figs. 14E, F, I; 15D) but *I. (P.)* cf. *aristaei* from Singapore has central teeth with well-defined, long, lateral margins that show some thickening and there is a prominent basal denticle on each side of each tooth (Fig. 15F). This type of central tooth is very similar to that seen in the genera *Liroceratia* nov., *Hyala* and *Nozeba* and it is similar to the central teeth of many rissoids and hydrobiids. It is thus probable that this type of tooth can be regarded as "primitive" and that the "typical" *Iravadia* central tooth is derived from it by a

loss of lateral thickening, a widening of the cutting edge and a reduction in the size of the basal denticles. This condition can be seen in *I. (P.) yendoi* (see Habe, 1958), a species almost indistinguishable from *I. (P.) aristaei,* in *I. (P.) densilabrum* (Fig. 14E, F) and in *I. (P.) bella* (Fig. 15D). A deep-water species from the Indian Ocean is closer to the type-species of *Pseudonoba* in shell features than *I. (P.) bella* and has a radula (Fig. 16F) in which the lateral edges of the central teeth are thickened and the cutting edge is narrow, giving the tooth a sub-triangular outline. There is a prominent basal denticle on the central tooth of this species but in a new species from the Philippines there are three to four large basal denticles (Fig. 16D, E). Both of these species are described in the Appendix. *I. (P.) densilabrum* has one to three rudimentary denticles, so that multiple basal denticles are not unique in the subgenus. The radula of two of the new species is also unusual in having a very prominent cusp on the lateral teeth and zero or one secondary cusp.

In view of the general similarity in the shell and opercular features, and the existence of intermediate radular types, the species are tentatively grouped into a single subgenus, *Pseudonoba.* It is probable, however, that when additional information is available, further division will be required.

Genus *Chevallieria* Cossmann, 1888: 244

Type-species: *Chevallieria labrosa* Cossmann, 1888; original designation. Eocene, Paris Basin, France.

Synonym: Nanadoma Laseron, 1956: 447. Type-species: *Nanadoma imitoris* Laseron, 1956; original designation. Recent, N Australia.

Diagnosis. **Shell.** Subcylindrical, thin, non-umbilicate, with convex whorls, basal fold weak to absent, smooth or with extremely fine spiral striae and, in some species, distinct axial growth lines. Aperture pyriform, angled and weakly channelled posteriorly, rounded to weakly angled anteriorly, outer lip orthocline to slightly opisthocline, varix strong to absent. Protoconch relatively large, flattened, smooth, of about 1¾–2 whorls. Fig. 17A–D, G, H. **Head-foot, penis** and **oviduct** unknown. **Operculum**. Elongate, with spiral form apparent; columellar edge slightly indented, transparent, a weak internal ridge along columellar edge (*C. australis* sp. nov.). Fig. 17E. **Radula**. Central teeth rather large, each with four small cusps on either side of a small median cusp; lateral margins unthickened, with a small denticle; basal margin with tongue-like projection. Lateral teeth with small, sharp cusps 5 + 1 + (?6); marginal teeth with numerous small, sharp cusps (*C. australis* sp. nov). Fig. 17F.

Distribution. Tropical Indo-Pacific (*N. imitoris; Rissoina columen* Melvill, 1904 = *Rissoina (Scrobs) elspethae* Melvill, 1910). New species from South Australia and the Miocene and Pliocene of Victoria, Australia are described in the Appendix. Eocene, Paris Basin (*C. labrosa* and *Chevallieria cylindroides* Cossmann, 1907). There have been several additional species attributed to *Chevallieria* from the Eocene of the Paris Basin, but these species have not been examined.

Habitat. The only species found alive (see Appendix) was collected under stones in a marine situation. *Chevallieria imitoris* also probably lives in a fully marine habitat.

Material Examined. C. labrosa. One specimen ex Cossmann colln. (NHMP), three specimens ex J. Le Renard (AMS). *N. imitoris.* Holotype, two paratypes and several other lots (AMS). *R. columen.* Holotype (BMNH). *R. (S.) elspethae.* Three syntypes (BMNH). *C. cylindroides.* One lot (AMS).

Remarks. The shell of the type-species of *Chevallieria* (Fig. 17A) differs from that of the type-species of *Nanadoma* (Fig. 17G) in having a weak basal fold and a strong varix on the outer lip. Another congeneric species found in the Eocene of the Paris Basin, *C. cylindroides,* lacks a basal fold and agrees extremely closely with a Miocene species from Victoria, Australia (Fig. 17B), which is described in the Appendix. This Australian species differs from *C. labrosa* mainly in having a weaker varix on the outer lip. The Recent *C. imitoris* has a very weak to absent varix.

Chevallieria labrosa closely resembles some species of *Iravadia (Pseudonoba)* in shell features and this genus is probably the group from which *Pseudonoba* evolved. Species of *Chevallieria* differ from those of *Pseudonoba* by their smaller, thinner shells and extremely delicate spiral sculpture, or smooth surface. The only operculum examined is similar to that of species of *Iravadia (Pseudonoba)* in shape but is more nearly spiral in construction, the nucleus being placed away from the margin (Fig. 17E). This type of operculum is intermediate between

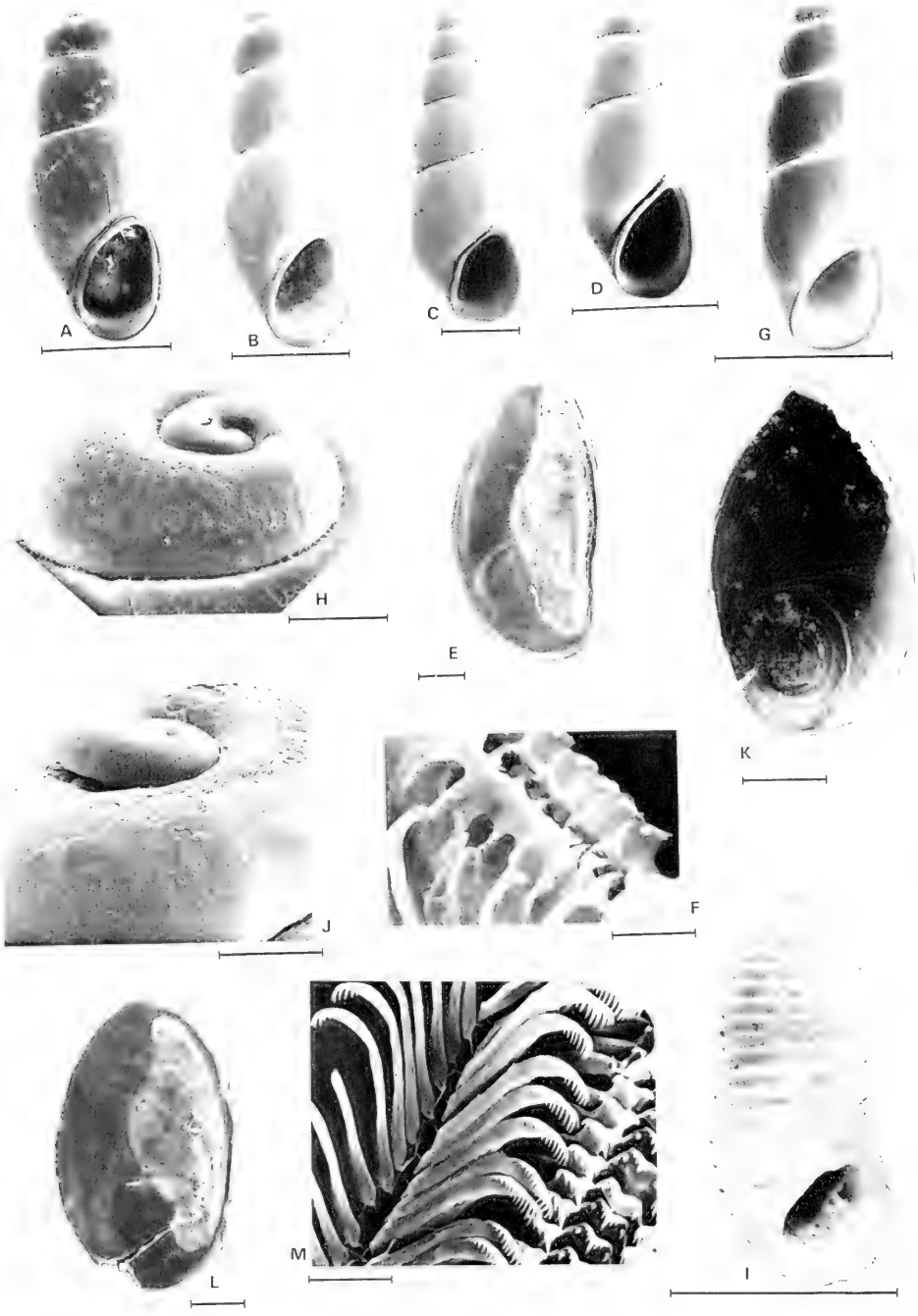

FIG. 17. A. *Chevallieria labrosa* Cossmann, type-species of *Chevallieria*; La Ferme de L'Orme, Yvelines, France (AMS). Shell. B. *Chevallieria balcombensis* sp. nov. Shell of holotype. C. *Chevallieria gippslandica* sp. nov. Shell of holotype. D–F *Chevallieria australis* sp. nov. Holotype. D. Shell. E. Operculum, inner side. F. Radula. G–H. *Chevallieria imitoris* (Laseron), type-species of *Nanadoma*, Shoal Bay, Mackay, Queensland, Australia (AMS). G. Shell. H. Protoconch. I–M. *Liroceratia sulcata* (Boettger), type-species of *Liroceratia* nov., Taurama, near Port Moresby, Papua, New Guinea (AMS). I. Shell. J. Protoconch. K, L. Operculum, outer side (K) and inner side (L). M. Radula. Scales: shells = 1 mm; opercula and protoconchs = 0.1 mm; radulae = 0.01 mm.

the normal spiral operculum seen in *Hyala, Nozeba* and *Liroceratia* and the typical *Iravadia* operculum. It is closer in form to the operculum of species of *Iravadia (Pseudonoba)* than to any of the other subgenera of *Iravadia*.

Genus *Rhombostoma* Seguenza, 1876: 14

Type-species: *Eulima carmelae* Brugnone, 1873; subsequent designation Sacco, 1892: 19. Pliocene, Sicily.

Synonym: Eulimopsis Brugnone, 1881: 120. Type-species: *Eulima carmelae* Brugnone, 1873; monotypy. Pliocene, Sicily.

Diagnosis. **Shell**. Small, elongate-conic, smooth or spirally sculptured, with weakly-convex whorls. Aperture elongately-ovate, angled and weakly channelled posteriorly; distinctly and rather deeply-channelled anteriorly; outer lip sinuate, with middle and uppermost (adapical) section advanced (see Fig. 13H). Inner lip thin, narrow; columella vertical, narrow. No external varix. Protoconch relatively small, of about two whorls, the first 1½ whorls flat, the last half whorl rapidly descending. Figs. 13E–H. **Animal** unknown.

Distribution. Pliocene and Miocene of Italy (*E. carmelae; Ondina imperforata* Sacco, 1892 = *Ondina pliobliqua* Sacco, 1892 = *striata auct.*). ?Miocene of Austria (*Chemnitzia striata* Hörnes, 1856).

Material Examined. E. carmelae. Syntypes (ZMR). *O. pliobliqua.* Sacco material (TGM), six specimens (ZMR). *C. striata.* Photograph of holotype *ex* A. Warén.

Remarks. Pavia (1975) has discussed this genus and describes in some detail one of the fossil species it contains. The shell of these species has a protoconch typical of the Iravadiidae (Fig. 13E) and a broad anterior notch in the aperture similar to that seen in some other genera in the family. The spiral cords of *R. imperforata* (Fig. 13E) also suggest a relationship with *Iravadia.* The weak varix and rather narrow aperture combined with the solid, tall-spired shell set the species included in this genus somewhat apart from the other genera in the family, although the overall apertural features resemble those of *Iravadia (Fairbankia) sakaguchii. Chemnitzia striata* Hörnes possibly belongs to this group although its aperture shows some similarity to that of species of *Iravadia (Pseudonoba)* in being less distinctly angled anteriorly.

Probably this group represents an off-shoot from an early *Pseudonoba* lineage but, because of its rather distinctive shell characters and the impossibility of confirming a close relationship with *Iravadia,* it is tentatively separated. Wenz (1940) and Pavia (1975) included this genus in the Eulimidae.

Genus *Liroceratia* Ponder, gen. nov.

Type-species: *Cingula sulcata* Boettger, 1893. Recent, Philippines.

Diagnosis. **Shell**. Small, solid, elongately-ovate, sculptured with strong spiral cords. Aperture oval, subangled posteriorly, rounded anteriorly, with weak, broad, anterior excavation; outer lip orthocline, with strong, broad varix. Protoconch similar to that of *Iravadia.* Periostracum yellow to brown, conspicuous. Fig. 17I, J. **Head-foot**. Cephalic tentacles long, strap-like, unpigmented, with stiff 'setae' distally; eyes at outer bases in small bulges. Snout of moderate length, bilobed. Foot weakly-cleft anteriorly, very weakly-indented posteriorly, anterior mucous gland indistinct. No posterior pedal mucous gland, no pallial tentacles and no metapodial tentacle. (*L. sulcata,* Taurama, near Port Moresby, Papua New Guinea). Fig. 1G. **Penis** and **oviduct** unknown. **Operculum**. Oval, coiled, thin, simple, nucleus eccentric, last whorl large. Fig. 17K, L. **Radula**. Very similar to that of *Hyala vitrea,* but with cutting edge of each lateral tooth raised on a neck-like extension from the base of the tooth. Fig. 17M.

Distribution. Tropical central Indo-Pacific from the Philippines to Fiji and Papua New Guinea (*C. sulcata, = Pellamora minatura* Laseron, 1956). *Rissoa truncata* Garrett, 1873 from Fiji is probably related.

Habitat. The seaward edge of mangroves under stones in the mid-littoral.

Material Examined. C. sulcata. Holotype (SMF), one lot Philippines and several other lots (AMS). *P. minatura.* Holotype and paratypes (AMS). *R. truncata.* Three syntypes (ANSP).

Remarks. This genus is based on a species which has a shell very like a miniature *Iravadia,* particularly in its protoconch characters and in having strong spiral cords. Its radula and operculum, however, resemble those of *Hyala vitrea.* The shell also superficially resembles that of marine species placed in rissoid genera such as *Lironoba* Iredale, 1915

but is easily distinguished by its protoconch characters (Fig. 17J), the rissoids having dome-shaped, sculptured protoconchs.

Species of *Chevallieria* are very similar to *Liroceratia sulcata* in their general shell features. They differ in their weak to absent spiral sculpture and in the details of the radular and opercular features of the one species for which these characters are known.

An Eocene species, *Ceratia (?) allixi* Cossmann, tentatively included in *Iravadia (Pseudonoba)* above, has similarities with this genus in most shell characters.

Genus *Hyala* H. & A. Adams, 1852: 359

Type-species: *Hyala vitrea (= Turbo vitreus* Montagu, 1803); monotypy. Recent, Europe.

Diagnosis. **Shell**. Small, thin, smooth, or with microscopic spiral threads. Aperture simple, oval, outer lip strongly prosocline, lacking a varix; weak posterior angulation present but no posterior sinus; shallowly but broadly excavated anteriorly. Protoconch smooth, of 2½ whorls, lacking a distinct terminal varix, first whorl rising slightly above level of nucleus. Fig. 18E–G. **Head-foot**. Animal unpigmented. Cephalic tentacles strap-like, long, having six to eight stationary cilia proximally, with eyes in the centre of their bases. Snout rather long, bilobed. Foot indented in front, rounded posteriorly. No accessory tentacles (*H. vitrea*, Clark, 1852 and A. Warén *in litt.,* 1981). Fretter & Graham (1978), apparently incorrectly, state that the posterior end of the foot is bifid. **Penis**. U-shaped when at rest, with short filament distally and small glandular bulge about two-thirds of length from base. Fig. 8E. **Oviduct**. With large anterior sperm sac, no bursa copulatrix and posterior oviducal opening. There are either one or two seminal receptacles. Fig. 3 and Johansson (1950). **Operculum**. Oval, thin, simple, spiral, with eccentric nucleus, last whorl very large (*H. vitrea*). Fig. 18H. **Radula**. Lateral and marginal teeth typical of family, central teeth with cutting edge relatively narrower than in *Iravadia,* cusps few, sharp, median cusp rather long; lateral margins spread outwards, slightly thickened; each tooth with a denticle on each side of face (*H. vitrea*). Fig. 18I, J.

Distribution. Coast of Europe and Mediterranean Sea (*H. vitrea = H. mediterranea* Nordsieck, 1972). Sea of Japan (? *H. adamsi* Golikov & Kussakin, 1971). Upper Oligocene, North Sea Basin (*Rissoa dissoluta* Wiechmann, 1874).

Habitat. Marine, sublittoral and on the continental shelf (15–100 m in deep burrows made by other animals on muddy bottoms (A. Josefson, personal communication, *fide* A. Warén *in litt.*) (*H. vitrea*). *Hyala adamsi* lives on *Zostera* in an enclosed bay with fluctuating salinity (Golikov & Kussakin, 1978).

Material Examined. H. vitrea. Several lots (BMNH, AMS and other museums). *R. dissoluta.* One lot (NMV).

Remarks. Thorson (1946) described a free-swimming larva from Denmark which he identified as "*Onoba vitrea.*" It had fine spiral striae on the only whorl of the teleoconch that had developed. He surmised that the smooth shell of specimens of *Hyala vitrea* was due to wear and that his larva belonged to that species. The shells examined in this study show no trace of spiral striae but Jeffreys (1867) and A. Warén (*in litt.*) state that very fine microscopic spiral striae are present in perfect specimens.

The type-species of the genus has a thin, smooth shell lacking a varix on the outer lip and has a paucispiral operculum. The somewhat flattened protoconch is similar to that of *Iravadia* and *Hyala* and also lacks a posterior mucous gland and accessory tentacles. In addition, the female reproductive system (Johansson, 1950) has a posterior slit in the glandular pallial duct, an anterior sperm sac, and lacks both a separate upper oviduct gland and a coiled oviduct, features also observed in *Iravadia* (herein).

The apertural features and general texture of the shell recall those of *Nozeba* and it is probable that these two genera are closely related. *Hyala vitrea,* however, differs from species of *Nozeba* in having a narrower shell, a strongly prosocline (not orthocline) outer lip and a weaker posterior angulation of the aperture.

Hyala adamsi has a much broader shell than *H. vitrea.* As I have not been able to examine specimens, the generic location of this species has not been confirmed. If it is a *Hyala*, a member of this genus can survive in waters of low salinity.

Genus *Ceratia* H. & A. Adams, 1852: 359

Type-species: *Rissoa proxima* (Alder MS) Forbes & Hanley, 1850; monotypy. Recent, Europe.

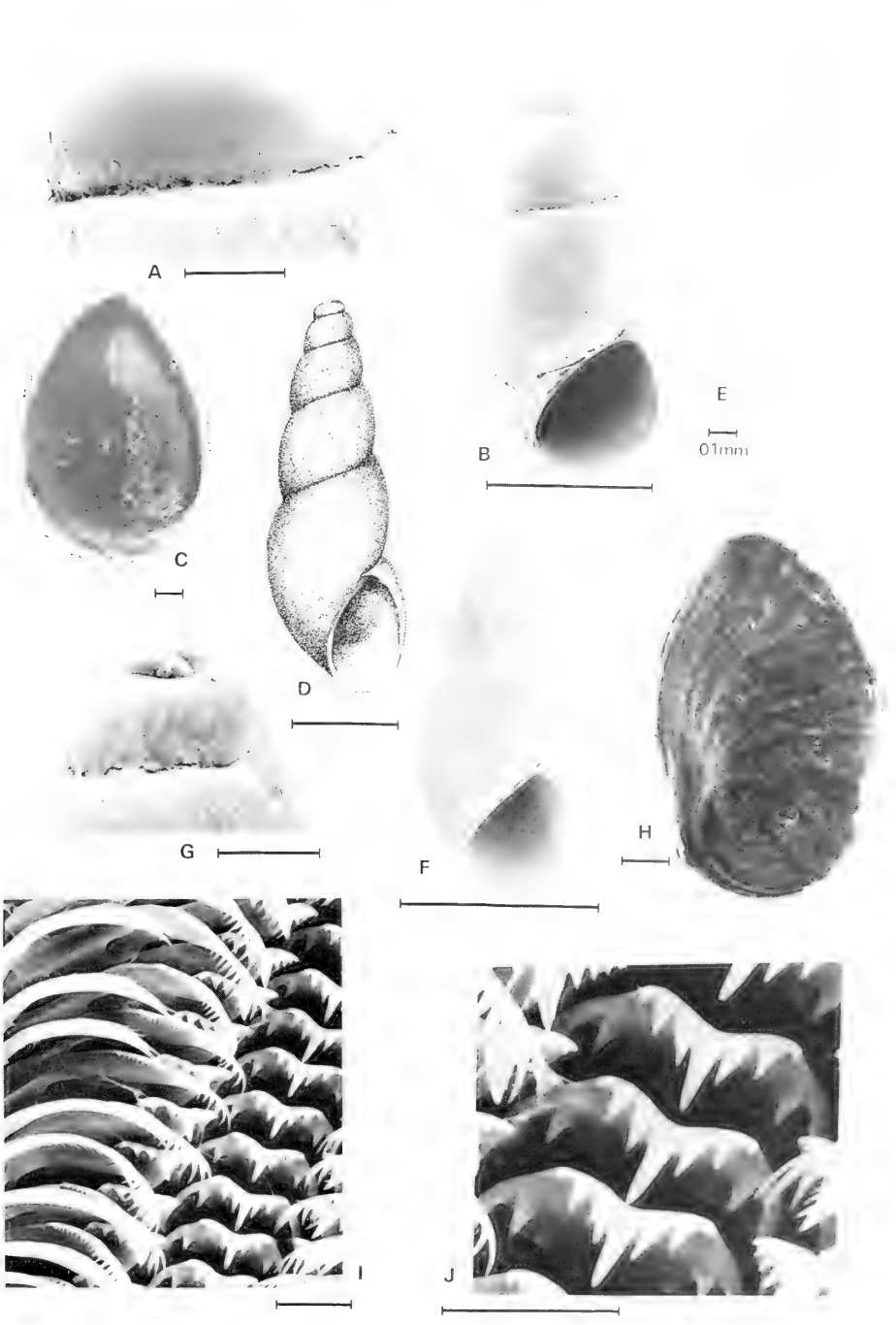

FIG. 18. A–C. *Ceratia proxima* (Forbes & Hanley), type-species of *Ceratia*. A. Protoconch. B. Shell. C. Operculum, inner side. A, B. Torbay, Devon, England (BMNH), C. Bay of Biscay, France (AMS). D. *Ceratia alta* (Gabb), type-species of *Hebetaclis*; holotype (ANSP). E–J. *Hyala vitrea* (Montagu), type-species of *Hyala*. E, F. Shell, Vigo Bay, Spain, 36 m (AMS). G. Protoconch, H. Operculum, outer side. I, J. Radula, J, central teeth. G–J. Horns Reef, Danish North Sea coast, 50 m (ZMC). Scales: shells 1 mm; opercula and protoconchs = 0.1 mm; radulae = 0.01 mm.

Synonym: Hebetaclis Pilsbry, 1922: 389. Type-species: *Auriculina alta* Gabb, 1873; original designation. "Miocene," Dominican Republic.

Diagnosis. **Shell**. Very similar to that of *Hyala* but with fine spiral striae. Protoconch of about two whorls, first whorl slightly elevated, nucleus depressed, similar to that of *Hyala*; smooth (except for "some fine spiral lines" according to Fretter & Graham, 1978). Fig. 18A, B, D. **Head-foot**. Pigmented white. Cephalic tentacles flat, rather short, smooth, gently attenuating and becoming minutely claviform at distal ends, which have a few stationary 'cilia'; eyes large, on minute swellings at outer bases of tentacles. Snout short but extendible, not bilobed. Foot large, fleshy, anterior edge deeply indented and produced into two long, lateral processes. Posterior end of foot divided into two long, widely divergent 'tails.' No accessory tentacles (Clark, 1852). **Penis** and **oviduct** unknown. **Operculum**. As in *H. vitrea (C. proxima)*. Fig. 18C. **Radula**. The only radula of *C. proxima* available was accidentally mounted upside down but the shape of the central tooth is more nearly square than in that of *H. vitrea*. Lateral teeth finely cuspate.

Distribution. Southern British Isles to the Mediterranean Sea (*Rissoa striatula* Jeffreys, 1847 (preocc.) = *C. proxima*.). *Ceratia proxima* has been recorded from the Pliocene of England (Jeffreys, 1867) and the Pliocene and Pleistocene of Italy (Pavia, 1975). Miocene, Dominican Republic (*A. alta*). *Ceratia minutissima* Cossmann, 1888 from the Eocene of the Paris Basin, is possibly congeneric but no specimens have been available for examination.

Habitat. Marine, subtidal and on the continental shelf (*C. proxima*).

Material Examined. R. striatula. Neotype (USNM). *C. proxima.* A few lots (BMNH, USNM). *A. alta.* Holotype and paratypes (ANSP).

Remarks. Ceratia is only tentatively regarded as a genus distinct from *Hyala* on the basis of the differences in the shape of the posterior end of the foot. Clark's (1852) description of the animal of *C. proxima* is based on the careful examination of at least two specimens. He observed this species together with *Hyala vitrea* and commented at length as to the distinctiveness of the animals. Certainly the deeply cleft posterior end of the foot of *C. proxima* appears to be unique and has been confirmed by Fretter & Graham (1978). Most species of *Iravadia* do, however, have the posterior end of the foot weakly-indented. Only one specimen containing a dried animal was available for examination and the mount of the radula was unsuccessful. The resuscitated dried remains did show that the posterior end of the foot was deeply cleft and that the operculum is identical to that of *H. vitrea*. Thus the familial position of this species is based mainly on shell features (which are almost identical to those of *Hyala*) and on the lack of acccessory tentacles.

Dr. A. Warén (*in litt.*) first examined the type of *A. alta* (Fig. 18D) and suggested its relationships. An examination of the type-material confirmed his assessment that *Hebetaclis* is a synonym of *Ceratia* as far as it is possible to judge from shell characters.

Genus *Nozeba* Iredale, 1915: 453

Type-species: *Rissoa emarginata* Hutton, 1885; original designation. Plio-Pleistocene and Recent, New Zealand. = *Neozeba, err. auct.*

[? = *Pasithea* Lea, 1833: 99, 207. Type-species: *Pasithea claibornensis* Lea, 1833; subsequent designation Gray, 1847: 160. Eocene, Alabama, North America. Synonym: ?*Pasitheola* Cossmann, 1896: 26. Unnecessary replacement name for *Pasithea* Lea, 1833, *non Pasythea* Lamouroux, 1812].

Synonyms: Antinodulus Cossmann, *in* Cossmann & Peyrot, 1919: 568. Type-species: *Bulimus globulus* Grateloup, 1827; original designation. Lower Miocene, France.

Syntharella Laseron, 1955: 100. Type-species: *Eulima topaziaca* Hedley, 1908; original designation. Recent, SE Australia.

Diagnosis. **Shell**. Ovate to elongately-conical, surface glossy, non-umbilicate, rather solid, smooth or with spiral threads, sometimes spirals moderately strong on base. Aperture rounded to distinctly-excavated anteriorly, sharply-angled posteriorly but not channelled. Posterior corner of aperture turned forward slightly, rest of outer lip approximately orthocline; inner lip partially disconnected from parietal wall in some species. No external varix. Protoconch smooth, of 2–2¼ convex whorls, flattened on top, last whorl descending, apex partially immersed. Periostracum thin. Fig. 19A, C–E, I. **Head-foot**. Cephalic tentacles long, strap-like, non-pigmented, with the eyes in small bulges at their outer bases; a few stiff "setae" distally.

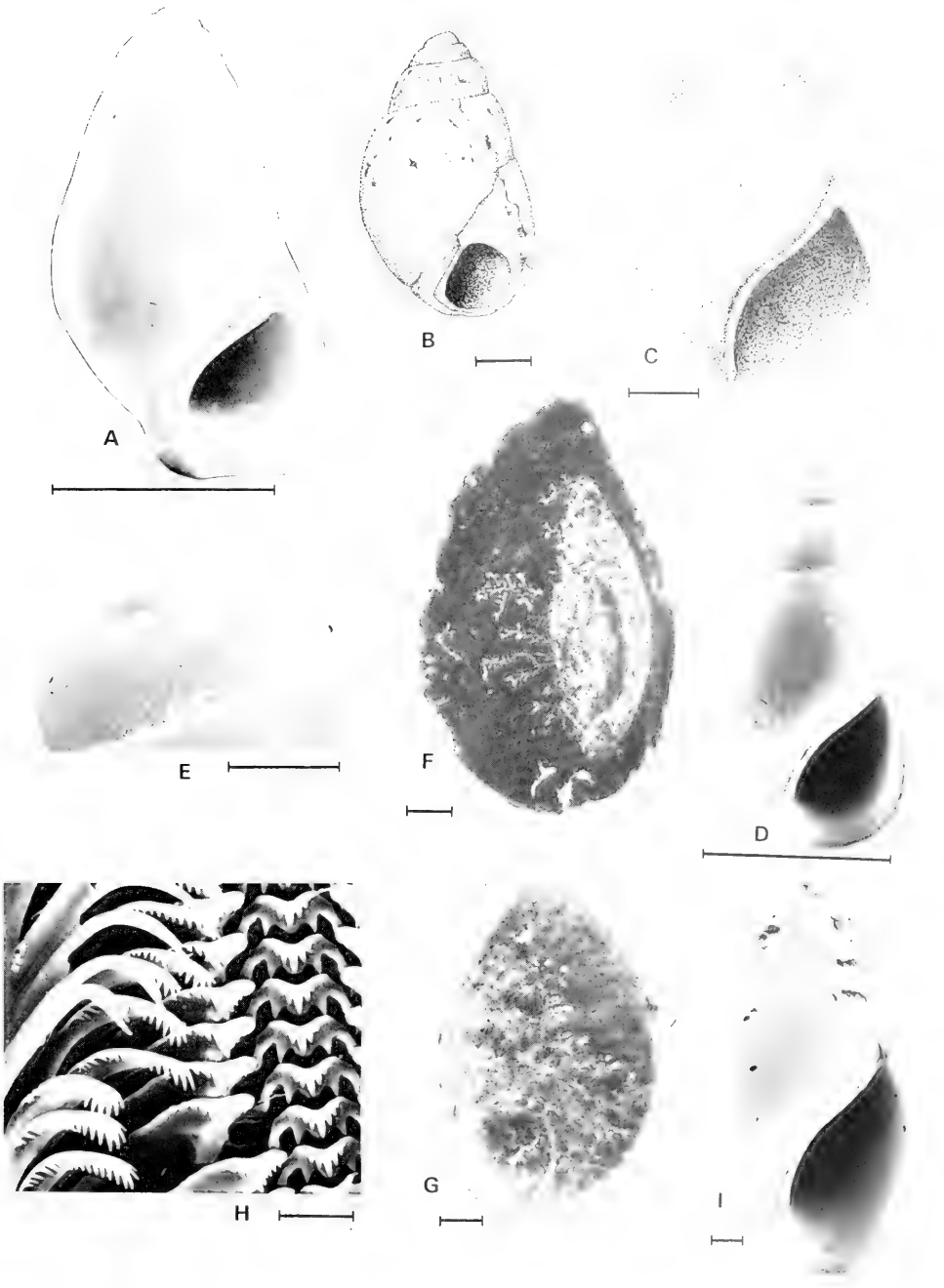

FIG. 19. A. *Nozeba emarginata* (Hutton), type-species of *Nozeba*. Outer Bay of Islands, New Zealand, 80 m (NMNZ). Shell. B. *Pasithea claibornensis* Lea, type-species of *Pasithea*. Shell of holotype. C. *Nozeba globulus* (Grateloup), type-species of *Antinodulus*; St. Paul-les-Dax, France (AMS). Shell. D–H. *Nozeba topaziaca* (Hedley), type-species of *Syntharella*. D. Shell. E. Protoconch. F, G. Operculum, inner (F) and outer (G) sides. H. Radula. D, F–H, Fisherman's Bay, Port Hacking, New South Wales, Australia (AMS); E. The Spit, Sydney, New South Wales (AMS). I. *Nozeba guttula* (Lea). Claiborne Sand Bed, Claiborne, Alabama, U.S.A. (USNM). Shell (subadult). Scales: shells = 1 mm; opercula and protoconch = 0.1 mm; radula = 0.01 mm.

Snout moderately long, extensile, bilobed. Foot slightly pigmented dorsally, with weakly-cleft anterior edge onto which opens a conspicuous, triangular anterior mucous gland; no posterior mucous gland present. Posterior pallial tentacle very short; no anterior pallial tentacle or metapodial tentacle. Posterodorsal side of foot simple. (*N. topaziaca*, Port Hacking, New South Wales). Fig. 1I. **Penis**. When at rest, bent double behind right cephalic tentacle, slightly to right of mid-line of head; wide, rather short, with enclosed duct opening at distal end, with three glandular swellings on inner edge. Fig. 8F. **Oviduct**. As for *Hyala*. **Operculum.** Oval, thin, simple, spiral, nucleus eccentric, last whorl large (*N. emarginata* Ponder, 1967; *N. mica, N. topaziaca*). Fig. 19F, G. **Radula**. Central teeth relatively large, cutting edge about equal to ½ total width, cusps small; lateral edges at about 45° to cutting edge, thickened, free from rest of base for most of their length. A pair of prominent denticles on face of each central tooth overlap tooth in front; a tongue-like extension of the base between. Lateral teeth elongate, cutting edge rather long, with many small cusps; a small protuberance on face of tooth below cutting edge. Marginal teeth long, curved, with many small cusps (*N. mica* Ponder, 1967; *N. topaziaca*). Fig. 19H.

Distribution. New Zealand, Recent (*R. emarginata* = *Rissoina coulthardi* Webster, 1908; *Nozeba mica* Finlay, 1930), Tertiary (Pliocene-Miocene) (four species—see Fleming, 1966). Temperate Australia (*Eulima topaziaca* Hedley, 1908 = *Estea amblycorymba* Cotton, 1944); Lower Miocene, Victoria (*Rissoa gatliffiana* Chapman & Gabriel, 1914). Eocene of France (*Amphimelania lucida* Cossmann, 1886; *Balanocochlis eulimoides* Cossmann, 1888). Eocene of Alabama, North America (*Pasithea guttula* Lea, 1833). Miocene of France (*B. globulus*).

A new species from the Philippine Islands is tentatively assigned to this genus and is described in the Appendix. Undescribed species have been seen from the Eocene of New Zealand and Upper Cretaceous (Ripley Formation) of the U.S.A.

Habitat. Marine, on the continental shelf (*N. mica, N. emarginata*) and living on sea grasses in estuaries and embayments in the lower littoral and sublittoral (*N. topaziaca*).

Material Examined. P. claibornensis. Holotype (ANSP). *R. emarginata.* A few lots (NMNZ, AMS). *N. mica.* A few lots (NMNZ). *E. topaziaca.* Holotype, paratypes and many other lots (AMS). *E. amblycorymba.* Holotype and other material identified by Cotton (SAM). *R. gatliffiana.* Three paratypes (NMV). *A. lucida* and *B. eulimoides.* One lot of each species, *ex* J. Le Renard (AMS). *P. guttula.* Holotype (ANSP) and two other lots (ANSP, USNM). *B. globulus.* One lot *ex* J. Le Renard (AMS).

Remarks. This genus has a long Tertiary history. The two Recent New Zealand species are marine but *N. topaziaca* from Australia lives in estuaries. A new species (see Appendix) tentatively referred to *Nozeba* was found in deep water in the Philippines. (Fig. 20G–J).

Pasithea may possibly be an earlier name for *Nozeba* but, unfortunately, that genus name is based on a very poorly preserved specimen which, according to Palmer (1937), may be a gerontic form of *P. guttula* (Fig. 19I). The type specimen (Fig. 19B) is, however, much larger than available material of *P. guttula* and may not be congeneric or even confamilial. *Pasithea guttula* is, together with some European Eocene species, included in *Pasitheola* by Cossmann (1921) and these certainly appear to be congeneric with *Nozeba.* An undescribed Eocene species from New Zealand is also a *Nozeba.* Gougerot & Le Renard (1977) have revised the Eocene species from the Paris Basin. Several specimens in the USNM from the Upper Cretaceous (Ripley Formation, SE U.S.A., USGS 25923, 28440, 27924) appear to be an undescribed species of *Nozeba.*

Cossmann, in several publications, considered the species he included in *Pasitheola* to be members of the Thiaridae. Other authors have placed this genus in the Eulimidae. *Nozeba topaziaca* (Fig. 19E–H), the type-species of *Syntharella* Laseron, agrees with *Nozeba emarginata* in essential shell characters and can be included in the genus *Nozeba. Syntharella* was regarded as a genus in the Eulimidae by Laseron (1955).

Genus *Rissopsis* Garrett, 1873: 228

Type-species: *R. typica* Garrett, 1873; monotypy. Recent, Fiji.

Diagnosis. **Shell**. Of moderate size, subcylindrical, thin, translucent, surface smooth and glossy, with flat whorls and pyriform aperture. Inner lip rather broad, thin; outer lip expanded slightly, orthocline to prosocline, aperture broadly excavated anteriorly; varix absent. Protoconch of about two whorls, very

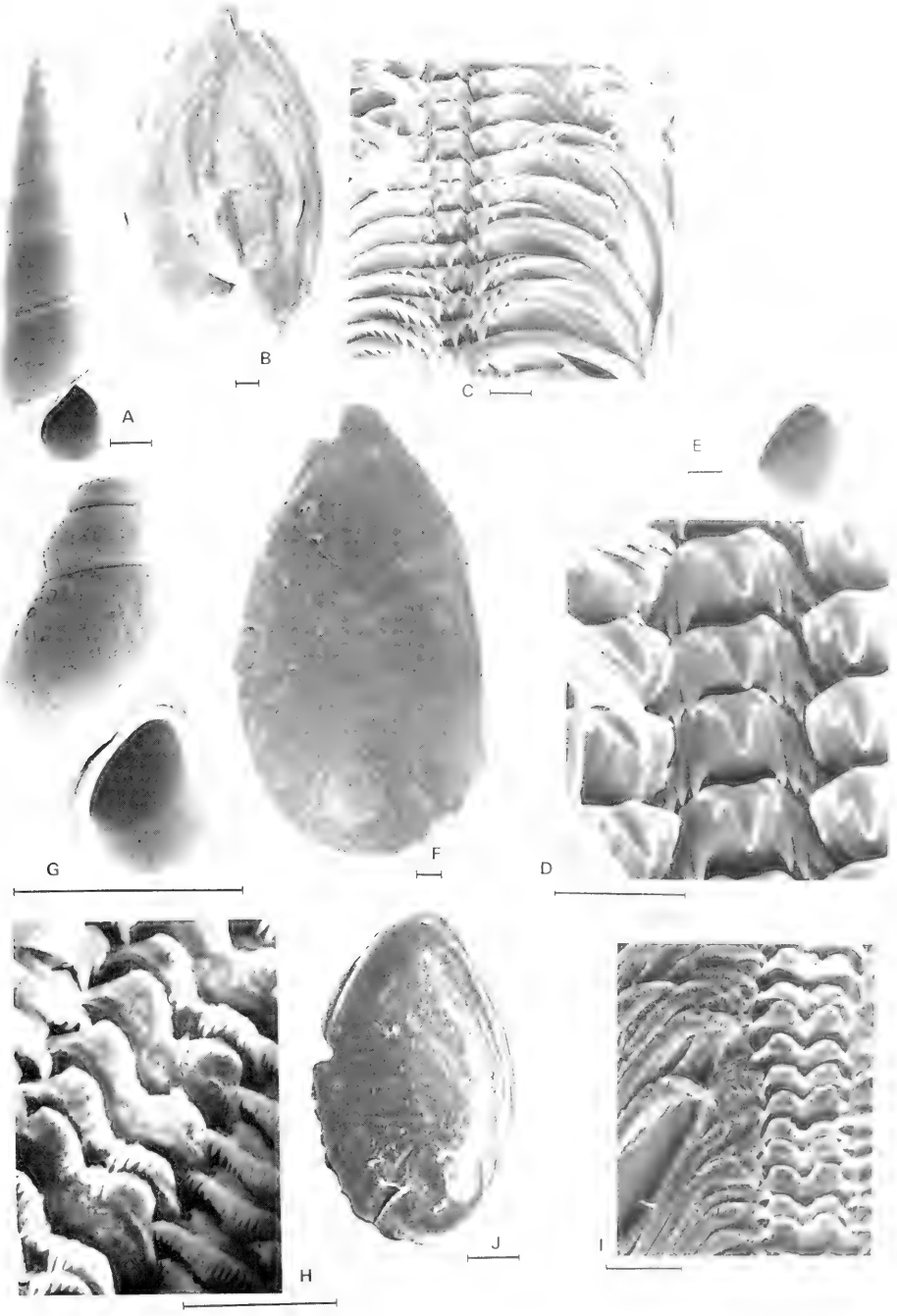

FIG. 20. A–D. *Acliceratia beddomei* (Dautzenberg), type-species of *Acliceratia* nov.; off Abidjan, Ivory Coast (NHMP). A. Shell. B. Operculum, inner side. C, D. Radula, D, central teeth. E–F. *Acliceratia carinata* (Smith): off Abidjan, Ivory Coast (NHMP). E. Shell. F. Operculum, inner side. G–J. *Nozeba (?) striata* sp. nov., holotype. G. Shell. H–I. Radula, H, central teeth. J. Operculum, inner side. Scales: shells = 1 mm; opercula = 0.1 mm; radulae = 0.01 mm.

small, tightly coiled, planorboid. (See Ponder, 1974, fig. 1). **Animal** unknown.

Distribution. Fiji, Samoa, Marshall Is. (*R. typica*); Durban, South Africa (*Rissopsis tuba* Kilburn, 1977; *Fusus prolongata* Turton, 1932 ? = *Rissopsis ligula* Kilburn, 1975). Northern Australia (one undescribed species). Philippines (one undescribed species).

Material Examined. R. typica. Lectotype and paralectotype (ANSP). One other specimen (SAM). *R. tuba.* Holotype and six paratypes (NM). *R. ligula.* Holotype and three paratypes (NM).

Habitat. Unknown.

Remarks. The type-species and the genus have been discussed by Ponder (1974). *Rissopsis* appears to be related to *Chevallieria* or, possibly, *Hyala*, judging from shell characters. Species of *Rissopsis* can be distinguished from those of *Chevallieria* by their large, smooth, glossy shell, the broadly-excavated anterior edge of the aperture and the subcylindrical spire. *Rissopsis* species resemble *Hyala vitrea* in having a smooth shell of similar shape, and, in *R. tuba*, a strongly prosocline outer lip. A more detailed assessment of the relationships of this genus must, however, await the examination of at least the radula and operculum.

Of the two species of *Rissopsis* from South Africa, one is distinct but the other (*R. ligula*) is very similar to *R. typica*, differing only in its slighty larger size. Single specimens of two undescribed species are known from northern Australia (AMS, C.126209) and the Philippines (USNM, 281302).

Genus *Acliceratia* Ponder, gen. nov.

Type-species: *Aclis beddomei* Dautzenberg, 1912. Recent, West Africa.

Diagnosis. **Shell**. Large for family, elongate-conic, thin, with spiral sculpture and a peripheral angulation or keel. Aperture angled posteriorly, rounded anteriorly; outer lip thin, lacking a varix, prosocline, with no posterior sinus; inner lip a thin, narrow glaze on parietal wall. Protoconch of about two whorls, typical of family. Fig. 20A, E. **Head-foot**. Unknown. Faecal pellets in rectum aligned perpendicular to rectal wall as is typical of family (*A. carinata*). **Penis** and **oviduct** unknown. **Operculum**. Thin, oval, simple, spiral, with eccentric nucleus, last whorl very large (*A. beddomei, A. carinata*). Fig. 20B, F. **Radula**. Central teeth sub-triangular, $\frac{(2)1 + 1 + 1(2)}{3 \qquad 3}$; primary cusp large, sharp; secondary cusps small; basal cusps prominent, sharp; outermost cusp on outer edge of tooth. Lateral teeth large, with long bases and prominent, pointed primary cusp; secondary cusps rather small, (3)2 + 1 + 5. Marginal teeth with sharp, rather large cusps, the largest about equal in size to primary cusps of central and lateral teeth (*A. beddomei, A. carinata*). Fig. 20C, D.

Distribution. West Africa (*A. beddomei; Aclis carinata* Smith, 1871). Paleocene, France (one undescribed species).

Habitat. On the continental shelf.

Material Examined. A beddomei. One lot (identified Dautzenberg) and one other lot (NHMP). *A carinata.* Two specimens Dautzenberg Colln.; IRSNB; one lot (NHMP). *A.* n. sp. One lot, *ex* Le Renard.

Remarks. Although the two Recent species of *Acliceratia* agree in opercular and apertural characters with *Ceratia* and *Hyala*, they differ in having a much larger shell (9–12 mm in length compared with about 3 mm for *Hyala vitrea* and *Ceratia proxima*) which bears a distinct peripheral ridge. The details of the central teeth of the radula of *C. proxima* are unknown but the very poor available mount shows them to be sub-rectangular, not sub-triangular as they are in *Acliceratia* species. The central teeth of *H. vitrea* are more similar in shape to those of *Acliceratia* but have only one pair of basal denticles. The two species of *Acliceratia* resemble in size and general shell characters some species included here in *Iravadia (Pseudonoba)* but they differ from all of the subgenera of *Iravadia* in having a simple, coiled operculum. The radula characters are similar to at least one species of *Iravadia (Pseudonoba)* in having multiple basal denticles on the central teeth. This character separates *Acliceratia* from the other genera (excluding *Ceratia* in which the details of the central teeth are not known) possessing a simple operculum.

The shells of the two Recent species (Figs. 20A, E) in this genus differ in size and in details of sculpture. They occur sympatrically and have almost identical radulae. It is possible that they represent morphs (sexual?) of a single species.

An undescribed Paleocene species from France came from shallow water deposits at Bachivillers, Oise. It agrees closely with the Recent species in shell characters.

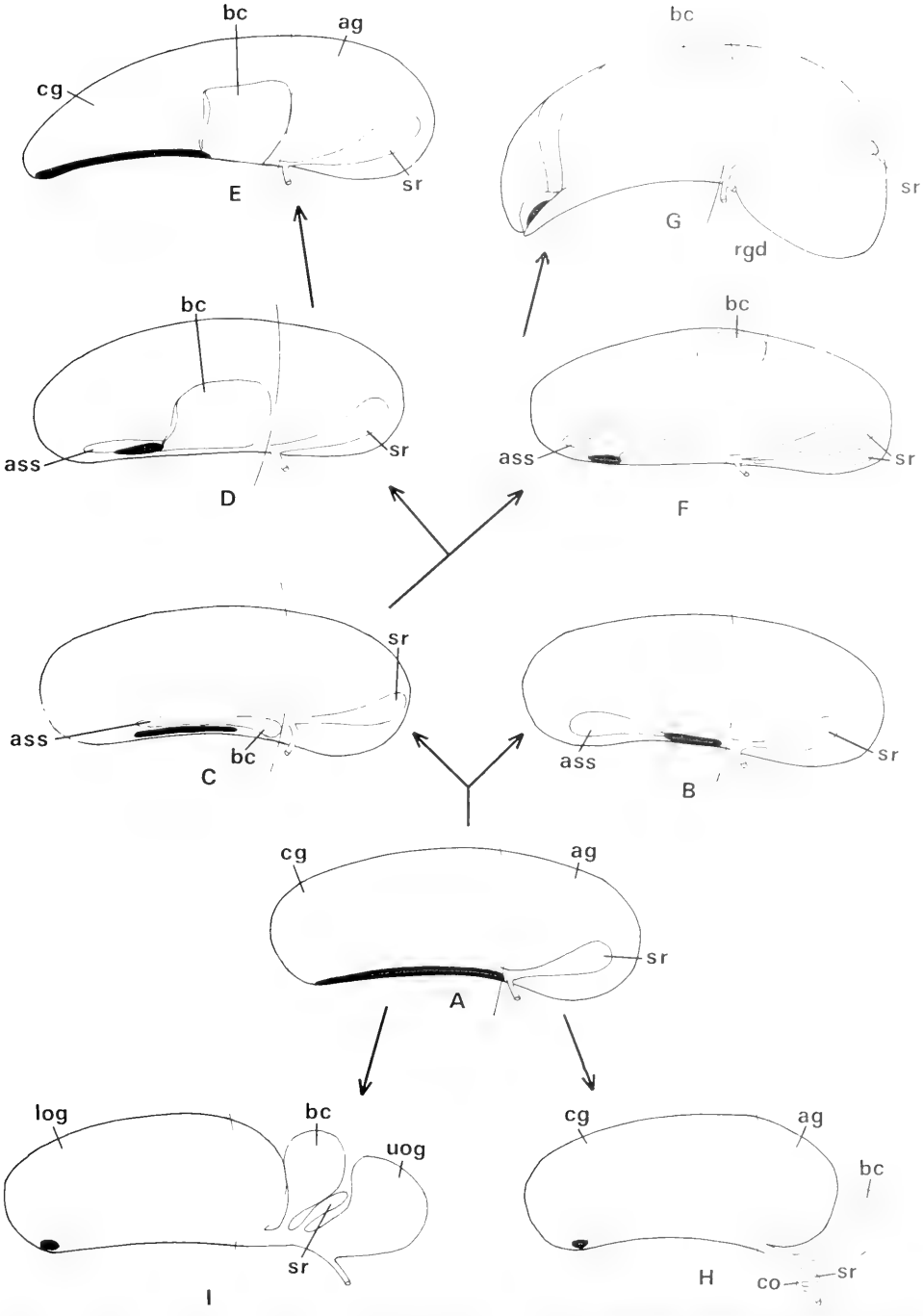

FIG. 21. Diagrams showing the types of female genitalia in the Iravadiidae and generalized examples of the Hydrobiidae and Rissoidae. The arrows indicate possible directions of evolutionary change in the genitalia and are not intended to indicate phylogenetic relationships. A. Hypothetical ancestral condition. B. *Hyala vitrea*. C. *Iravadia (Iravadia) quadrasi*. D. *Iravadia (Fairbankia) australis*. E. *Iravadia (Fairbankia) bombayana*. F. *Iravadia (Pseudomerelina) mahimensis*. G. *Iravadia (Iravadia) ornata*. H. Generalized Hydrobiidae. I. Generalized Rissoidae.

DISCUSSION

Evolution of the Iravadiidae

Female Genitalia. A hypothetical evolutionary scheme of the female genital system is schematically shown in Fig. 21. Here it is assumed, following Johansson (1968), that the ancestral condition was an open pallial oviduct (Fig. 21A). Closure of the originally open capsule gland from behind forwards, enclosing the sperm groove within the ventral channel, apparently occurred in the Hydrobiidae (Johansson, 1948) (Fig. 21H). It appears as though, in the Iravadiidae, closure of the ventral opening occurred differently, beginning anteriorly and moving backwards. This resulted in a slit-like opening in the posterior half of the capsule gland as seen in *Hyala vitrea* amd *Nozeba topaziaca* (Fig. 21B). Further evolution appears to have resulted in the opening becoming secondarily anterior (as in *Iravadia ornata*) (Fig. 21G) or even, apparently secondarily, open over much of its length (as in *Iravadia (Fairbankia) bombayana)* (Fig. 21E). A sperm sac (ass) developed anterior to the opening by the sperm groove, closing over to become a tubular structure. Posteriorly, in *Iravadia quadrasi* (Fig. 21C), the deep, muscular sperm groove is open throughout the posterior part of the oviducal opening and contains unorientated sperm. Just posterior to the oviducal opening the sperm groove closes over, becoming a blind sac (bc) which extends to the posterior end of the pallial cavity. This sac, or bursa copulatrix, develops in a similar way in the other species of *Iravadia* investigated, although its relative position and its opening vary considerably. In the subgenus *Fairbankia* the bursa and its opening have swung dorsally to form a lateral pocket-like structure with a separate pallial opening. *Iravadia (Fairbankia) australis* (Fig. 21D) retains a rudimentary anterior sperm sac but this is lost in *I. (F.) bombayana* (Fig. 21E), in which the anterior two-thirds of the capsule gland is open ventrally. In *Iravadia (Pseudomerelina) mahimensis* (Fig. 21F), the bursal opening is enclosed immediately behind the oviducal opening. It appears as though the bursa (bc) in this species has migrated dorsally and the opening of the bursal duct has retained its original position. The bursal duct is developed from part of the sperm groove, whereas the bursa itself is probably an outgrowth from this groove. The bursal sac has migrated back behind the pallial cavity and lies on the outer side of the glandular oviduct in *Iravadia ornata* (Fig. 21G). In that species the anterior sperm sac is apparently reduced to a small vestibule-like structure (although the sperm storing function of this area was not confirmed) and the bursal duct runs almost vertically from just behind the short, subterminal genital opening.

Operculum. The peculiar operculum of *Iravadia* (s.l.) is a considerable departure from that of the normal, coiled, paucispiral operculum seen in the other genera of the Iravadiidae and in most Rissoidae and Hydrobiidae. The modified operculum may have arisen in a *Chevallieria* ancestor in the early Tertiary as discussed above (see Remarks under *Iravadia* and *Chevallieria*, and Fig. 22). It is assumed that this type of operculum has arisen only once and that *Iravadia*, as here recognised, is a monophyletic group (Fig. 23).

Radula. The assumption that the marine genera are the most primitive is supported by species of *Hyala* and *Nozeba* having a single pair of well-developed denticles on the face of the central teeth of their radulae, this being the normal rissoacean condition. This character is shared by the estuarine genus *Liroceratia* and these three genera are similar in having a non-pigmented animal and a normal, spirally-coiled operculum. An increase in the number of basal denticles on the central teeth of the radula may have occurred more than once, several pairs being found in *Acliceratia* and some species of *Iravadia* (s.l.). The reduction and, sometimes, eventual loss of denticles on the face of the central teeth of the radula, due to their lateral migration, appears to be a common trend in the genus *Iravadia* (s.l.).

Habitat. Species of *Chevallieria* appear to be marine and the genus, like *Nozeba*, has an ancestry extending back at least to the Eocene. *Chevallieria* is probably ancestral to *Iravadia (Pseudonoba)*, which is recorded from the Miocene. Some species of the *Pseudonoba* group migrated into brackish water and appear to have given rise to the other subgenera of *Iravadia*, all of which are confined to waters of reduced salinity. Other incursions into brackish water have been made by at least one species in both *Nozeba* and *Liroceratia* (Fig. 22) and, possibly, *Hyala*. This propensity for the iravadiids to move into low salinity areas might suggest that the ancestral group inhabited sheltered bays and estuaries and that the marine species in the family are derived from these. A detailed an-

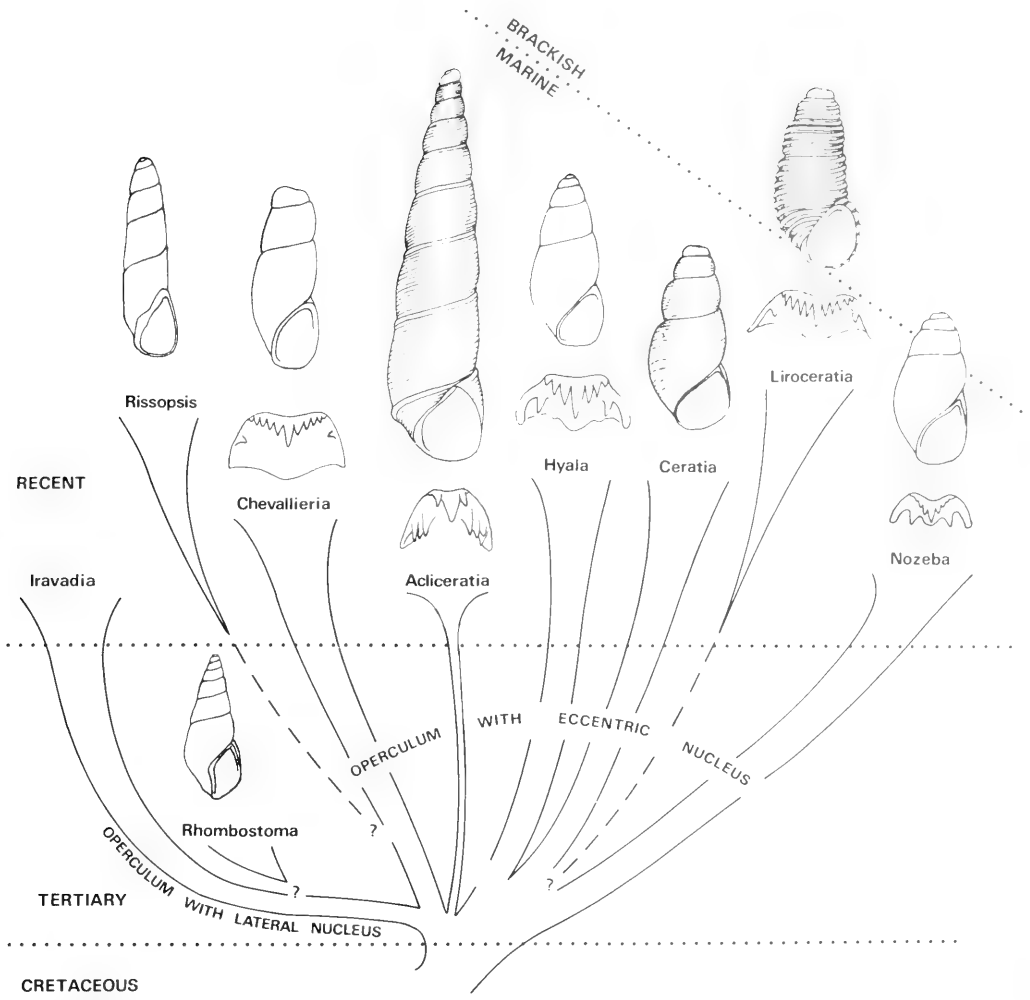

FIG. 22. A speculative interpretation of the evolution of genera of the Iravadiidae through time and their incursions into brackish water. The diagram is intended to indicate possible relationships and display shell diversity and the differences in the structure of the central teeth of the radula.

alysis of the habitats of the older fossil species has not been possible but the indications are that they are found together with typically marine species. The assumed movement into low salinity areas, mainly mangrove habitats, by the ancestral *Iravadia* stock appears to have been followed by a minor adaptive radiation resulting in a wide diversity of shell form and sculpture (Fig. 23). Species included here in the subgenus *Pseudonoba* range from brackish-water mangrove habitats to fully marine, relatively deep-water species.

Classification

In general, less is known about the genera possessing a spiral operculum than *Iravadia*. They appear to be generally related and have many of the characters listed in Table 2 in common. There is, however, little justification or advantage, considering the available evidence, for merging them to any greater extent than has been done, until more about them is known. It is possible that at least *Hyala* and *Ceratia* may prove to be congeneric but the other groups probably represent lineages that have been long separated and are thus probably anatomically distinct. Fig. 22 is an attempt to indicate probable relationships and provide a summary of my concept of the evolution of the group. Two genera (*Rissopsis* and *Rhombostoma*) are known only from their shells, so that their detailed relationships are

TABLE 2. A list of some of the morphological and habitat features used to distinguish the fourteen genus-group taxa of the Iravadiidae (+, present in all species; ±, present in some species; −, absent in all species; ++ very strongly developed; (+), very poorly developed).

	Iravadia	*Iravadia* s.s.	*Pseudomerelina*	*Fairbankia*	*Fluviocingula*	*Pseudonoba*	*Rhombostoma*	*Chevallieria*	*Hyala*	*Ceratia*	*Acliceratia*	*Liroceratia*	*Nozeba*	*Rissopsis*
1. Pallial tentacle present	−	−	−	−	−	−	?	?	−	−	?	−	+	?
2. Metapodial tentacle present	−	−	+	−	−	−	?	?	−	−	?	−	−	?
3. Cephalic tentacles pigmented	±	+	+	+	+	−	?	?	−	−	?	−	−	?
4. Posterior end of foot														
A. Bifid	−	−	−	−	−	−	?	?	−	+	?	−	−	?
B. Rounded or weakly indented	+	+	+	+	+	+	?	?	+	−	?	+	+	?
5. Basal cusps on central radular teeth														
A. One pair	±	±	+	−	±	±	?	+	+	?	−	+	+	?
B. 2–4 pairs	±	−	−	+	±	±	?	−	−	?	+	−	−	?
C. None	±	±	−	−	±	±	?	−	−	?	−	−	−	?
6. Operculum														
A. Nucleus marginal	+	+	+	+	+	+	?	−	−	−	−	−	−	?
B. Nucleus submarginal	−	−	−	−	−	−	?	+	−	−	−	−	−	?
C. Nucleus eccentric	−	−	−	−	−	−	?	−	+	+	+	+	+	?
7. Shell sculpture														
A. Spirals predominant	±	+	+	±	−	−	±	−	−	−	+	+	−	−
B. Smooth or with weak spiral threads	±	−	−	±	−	+	−	+	+	+	−	−	+	+
C. Axials prominent	±	±	+	−	−	−	-				-	-	-	−
D. With weak axial and spiral threads	±	−	−	−	+	±	−	−	−	−	−	-	−	-
8. Aperture of shell with anterior angulation	±	+	−	+	+	+	++	±	+	+	+	−	+	+
9. Outer lip of aperture														
A. Opisthocline	(+)	−	−	−	−	(+)	−	(+)	-	−	−		−	−
B. Orthocline	±	−	-	−	−	±	±	+	−	−	−	+	+	±
C. Prosocline	±	+	+	+	+	±	+	−	+	+	+	−	(+)	±
10. Bursa copulatrix														
A. Dorsal and pallial	±	−	+	−	?	?	?	?	−	?	?	?	−	?
B. Dorsal, behind pallial cavity	±	±	−	−	?	?	?	?	−	?	?	?	−	?
C. Ventral and pallial	±	±	−	−	?	?	?	?	−	?	?	?	−	?
D. Lateral and pallial	±	−	−	+	?	?	?	?	−	?	?	?	−	?
E. Absent	−	−	−	−	?	?	?	?	+	?	?	?	+	?
11. Anterior sperm sac														
A. Well-developed	±	±	−	−	?	?	?	?	+	?	?	?	+	?
B. Poorly-developed	±	±	+	±	?	?	?	?	−	?	?	?	−	?
C. Absent	±	−	−	±	?	?	?	?	−	?	?	?	−	?
12. Habitat														
A. Marine	±	−	−	−	−	±	?+	+	+	+	+	−	±	+
B. Estuarine	±	+	+	+	+	±	?−	−	(+?)	−	−	+	±	−

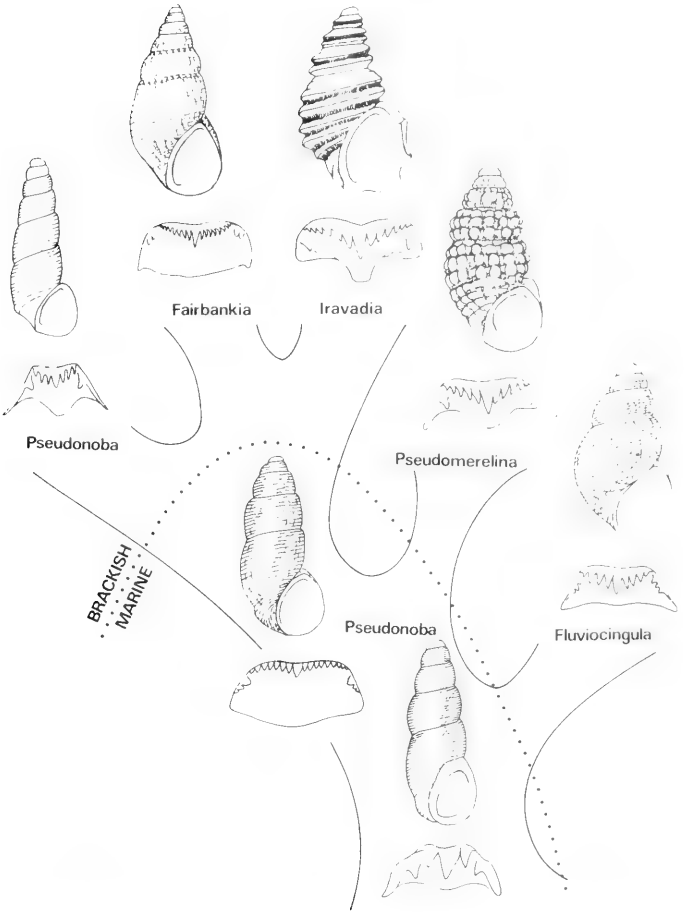

FIG. 23. Diagram showing diversity of shell morphology and the shape of the central teeth of the radula in the genus *Iravadia*. In addition, speculative evolutionary relationships and incursions into brackish water are indicated.

impossible to assess. *Hyala vitrea* is known in reasonable detail and only one, possibly atypical, species of *Nozeba* has been investigated anatomically. The head-foot of the type-species of *Liroceratia* and *Ceratia* is known and only the radula and operculum of one species of *Chevallieria* have been available for examination. Thus, the conclusions made about the relationships of these genera must be considered tentative.

The subdivision of *Iravadia* is more conservative than that of previous classifications because of the considerable similarity of the observed characters, with the exception of the shell characters in some cases. The genitalia also show differences but there are limitations in the knowledge of the structure of the female genital system, in particular, for some of the groups. The grouping within *Iravadia* assumed to be the most primitive (*Pseudonoba*), contains species showing a wide diversity of radular structure and habitat and may be subdivisible. Any refinement of the classification, however, seems inadvisable until more evidence is available.

Familial Relationships

Anatomically the Iravadiidae are very similar to the Hydrobiidae (as defined by Davis, 1980) but the shells have more resemblance to those of rissoids. Both the Rissoidae and an expanded concept of the Hydrobiidae (see below) are contrasted in Table 1 with the Iravadiidae.

Radoman (1973) has defined nine families in his "superfamily" Hydrobioidea. On the available information these all appear to be

closer to the Hydrobiidae than to the Rissoidae, Iravadiidae or Pomatiopsidae. For this reason the table of characters comprising the Rissoidae, Hydrobiidae and Iravadiidae includes the characters of all Radoman's Hydrobioidea, as defined by him, combined with the definition of the Hydrobiidae of Davis (1980). This grouping is loosely referred to as Hydrobiidae *sensu lato*.

The considerable similarity in the structure of the head-foot, the nervous system, the alimentary canal and the male genital system in the Iravadiidae and the Hydrobiidae (s.l.) may be due to convergence but there is also a strong possibility that the two groups may have a common ancestry.

The peculiar, flattened, glossy protoconch is the only uniform and distinctive shell feature which characterises the Iravadiidae. The iravadiids investigated apparently lack a distinct posterior pedal gland and, in most species, a metapodial tentacle and pallial tentacles. The peculiar penes seen in this group are reminiscent of some Hydrobiidae in having accessory, glandular swellings. This feature is also seen in some species of *Rissoina* (Kosuge, 1965; Ponder, 1968) but is otherwise unknown in the Rissoidae. The long, coiled dorsal folds in the anterior section of the oesophagus are other structures which are shared with the Hydrobiidae.

Johansson (1950) described the female reproductive system of *Hyala vitrea* (Montagu) and showed that it had an anterior sperm sac and that the slit-like opening to the female genital duct was near the posterior end of the glandular pallial duct. Johansson doubted that *Hyala vitrea* should be included in the Rissoidae but, in the latest revision of the European rissoids (Fretter & Graham, 1978), *H. vitrea* is, along with *Ceratia proxima*, included in *Onoba* Adams, the type-species of which (*O. semicostata* (Montagu)) is known to have a normal rissoid female genital system (Fretter & Patil, 1961).

The Iravadiidae and Hydrobiidae (s.l.) agree in most anatomical features except for the lack of spherules in the digestive gland of the Iravadiidae and in the structure of the female genital duct. They also differ from each other in their protoconchs; most hydrobiids having dome-shaped protoconchs of about 1½ whorls with an irregularly pitted surface. This is in sharp contrast to the small, somewhat planorboid, smooth protoconchs of species of the Iravadiidae.

The protoconch differences may, however, be partly due to the assumed different developmental modes in the two families. The veliger larvae of *Hyala vitrea* are known to be planktonic (Thorson, 1946) and, although there are no direct observations on the other species in the family, the morphology of their protoconchs strongly supports the view that they all have planktotrophic larvae. Hydrobiids, on the other hand, are probably all direct developers.

The lack of spherules in the digestive gland is unusual in the Rissoacea and seems to be a constant feature, as determined histologically. These spherules have been consistently observed in other rissoacean families (my own observations, Fretter & Graham, 1962) except the Assimineidae.

The female reproductive system of *Iravadia ornata* superficially resembles that of members of the Hydrobiidae. Common features include the small, subterminal genital opening and the bursa copulatrix lying, in part, behind the pallial cavity. *Iravadia ornata* differs from the hydrobiids in having a pallial bursal duct, in much of the bursa lying along the capsule gland, in lacking a narrow, coiled section of the oviduct between the renal oviduct and the oviduct gland, and in having a large seminal receptacle which opens at the junction of the ventral channel and the renal oviduct. It is conceivable that the female genitalia characteristic of the Hydrobiidae evolved from an iravadiid similar to *I. ornata*. This would have to occur by the posterior migration of the opening to the bursal duct because the bursa copulatrix of the hydrobiids, and its duct, lie behind the pallial cavity where they enter an extension of the ventral channel beneath the albumen gland. This sequence of events, however, is unlikely because of the specialized opercula in *Iravadia* species and the lack of an oviducal coil.

The longitudinal fold in the ventral channel of the posterior end of the capsule gland and the anterior part of the albumen gland of the iravadiids is probably homologous to the almost identical fold in the ventral channel of the capsule gland of the Hydrobiidae. In that family, however, the albumen gland lacks a ventral channel, this continuing posteriorly from the capsule gland as a closed, separate tube. The ventral channel in the Rissoidae lacks a fold (Johansson, 1948; Fretter & Graham, 1962). The homology of this fold is clear in *Hyala vitrea* (Fig. 3, lf) where the sperm groove (sg) lies behind the fold and continues posteriorly to the opening of the seminal

receptacle. The situation in the genus *Iravadia* (Figs. 4–7) is somewhat complicated by the formation of a secondary fold and groove to a greater or lesser extent within the sperm groove and the derivation of the bursa copulatrix from the sperm groove. The fold persisting to the entrance of the seminal receptacle in *Iravadia* species appears to be derived from the upper inner edge of a "greater sperm groove" (i.e., the secondary grooves are apparently derivatives of the originally single sperm groove) and can thus be regarded as homologous with the fold in *Hyala* and the hydrobiids.

The bursa copulatrix (as here recognised) of the Iravadiidae is possibly not homologous with that of the Hydrobiidae and Rissoidae. The bursa in these families agrees in position with the iravadiid seminal receptacle and may be homologous with it. The seminal receptacle(s), in the Hydrobiidae, in particular, usually opens into the coiled section of the oviduct and may be a new structure.

The Pomatiopsidae have been described and defined by Davis (1979, 1980) and show a wide variety of shell characters. All of the members of this family have two openings to the female genital system, a distal oviducal opening and a spermathecal opening. A narrow spermathecal duct leads to the bursa copulatrix, which lies behind the pallial cavity. The radula also differs; the innermost basal cusps on the central teeth are larger than the outer ones, the opposite condition to that seen in the Iravadiidae.

An anterior sperm sac (or bursa copulatrix) is known in two other rissoacean families, the Caecidae (Marcus & Marcus, 1963), which have uncoiled shells, and the Vitrinellidae (Fretter, 1956), which have depressed-helicoid shells and animals with well developed pallial and metapodial tentacles.

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REFERENCES CITED

ADAMS, H. & ADAMS, A., 1852, On a new arrangement of British Rissoae. *Annals & Magazine of Natural History,* (2)10: 358–359.

ANNANDALE, N. & PRASHAD, B., 1919, Some gastropod molluscs from the Gangetic delta. *Records of the Indian Museum,* 16: 241–257, 1 pl.

BLANFORD, W. T., 1867, Contributions to Indian malacology No. 8. List of estuary shells collected in the Irawadi in Pegu, with descriptions of new species. *Journal of the Asiatic Society of Bengal,* 36: 51–72.

BLANFORD, W. T., 1868, Descriptions of *Fairbankia*, a new genus and species of Rissoidae from Western India. *Annals & Magazine of Natural History,* (4)2: 399–401.

BOETTGER, O., 1902, Zur Kenntnis der fauna der mittelmiocänen Schichten von Kostej im Krasso-Szorényer Komitat. II. *Verhandlungen und Mitteilungen des Siebenburgischen Vereins für Naturwissenschaften in Hermannstadt,* 51: 1–200.

BRANDT, R. A. M., 1968, Description of new non-marine mollusks from Asia. *Archiv für Molluskenkunde,* 98: 213–289.

BRANDT, R. A. M., 1974, The non-marine aquatic Mollusca of Thailand. *Archiv für Molluskenkunde,* 105: i–iv, 1–423.

BRUGNONE, G., 1881, Le conchiglie Plioceniche delle vicinanze di Caltanisetta. *Bullettino della Società Malacologica Italiana*, 6: 83–157, 1 pl.

CLARK, W., 1852, On some undescribed animals of the British Rissoae. *Annals & Magazine of Natural History*, 10: 254–264.

COAN, E., 1964, A proposed revision of the Rissoacean families Rissoidae, Rissoinidae, and Cingulopsidae (Mollusca: Gastropoda). *Veliger*, 6: 164–171.

COSSMANN, M., 1888, 1896, Catalogue illustré des Coquilles Fossiles de l'Eocène des environs de Paris. *Annales de la Société royale malacologique de Belgique*, 23(1888): 1–328, pl. 1–12; 31(1896): 1–94, pl. 1–3.

COSSMANN, M., 1921, *Essais de Paléoconchologie Comparée.* Livr. 12: 1–336, pl. 1–11. Paris.

COSSMANN, M. & PEYROT, A., 1916–1919, Conchologie néogénique de l'Aquitaine. Tome III (Gastropodes). *Actes de la Société Linnéenne de Bordeaux,* 69: 157–365; 70: 5–212.

DANCE, S. P. & EAMES, F. E., 1966, New molluscs from the Recent Hammar Formation of south-east Iraq. *Proceedings of the Malacological Society of London,* 37: 35–43.

DAVIS, G. M., 1979, The origin and evolution of the gastropod family Pomatiopsidae, with emphasis on the Mekong River Triculinae. *Academy of Natural Sciences of Philadelphia, Monograph* 20: 1–120.

DAVIS, G. M., 1980, Snail hosts of Asian *Schistosoma* infecting man: evolution and coevolution. *In* The Mekong Schistosome; Malacological Review, Supplement 2: 195–238.

FLEMING, C. A., 1966, Marwick's illustrations of New Zealand shells with a checklist of New Zealand Cenozoic Mollusca. *New Zealand Department of Scientific & Industrial Research, Palaeontological Bulletin,* 173: 1–456.

FRETTER, V., 1956, The anatomy of the prosobranch *Circulus striatus* (Philippi) and a review of its systematic position. *Proceedings of the Zoological Society of London,* 126: 369–381.

FRETTER, V. & GRAHAM, A., 1962, *British Prosobranch Molluscs, their functional anatomy and ecology.* Ray Society, London, xvi + 755 p.

FRETTER, V. & GRAHAM, A., 1978, The prosobranch molluscs of Britain and Denmark. Part 4—Marine Rissoacea. *Journal of Molluscan Studies, Supplement* 6: 151–241.

FRETTER, V. & PATIL, A. M., 1961, Observations on some British rissoaceans and a record of *Setia inflata* Monterosato, new to British waters. *Proceedings of the Malacological Society of London,* 34: 212–223.

GARRETT, A., 1873, Descriptions of new species of marine shells inhabiting the South Sea Islands. *Proceedings of the Academy of Natural Sciences of Philadelphia,* 1873: 209–231, 233–237.

GOLIKOV, A. N. & KUSSAKIN, O. G., 1978, *Shell bearing littoral gastropods of the seas of the U.S.S.R.* Leningrad, 256 p., 33 pl. (in Russian).

GOLIKOV, A. N. & STAROBOGATOV, Ya. I., 1975, Systematics of prosobranch gastropods. *Malacologia,* 15: 185–232.

GOUGEROT, L. & LE RENARD, J., 1977, Clefs de détermination des petites espèces de gastéropodes de l'Eocène du Bassin Parisien. VIII. Le genre *Graphis*; IX. Le genre *Pasitheola. Cahiers des Naturalistes. Bulletin des Naturalistes Parisiens,* 30: 37–54.

GRAY, J. E., 1847, A list of the genera of Recent Mollusca, their synonyma and types. *Proceedings of the Zoological Society of London,* 15: 129–219.

HABE, T., 1958, On the radulae of Japanese marine gastropods (4). *Venus, Japanese Journal of Malacology,* 20: 43–60.

IREDALE, T., 1915, A commentary on Suter's "Manual of the New Zealand Mollusca." *Transactions & Proceedings of the New Zealand Institute,* 47: 417–497.

IREDALE, T., 1943, A basic list of the fresh water Mollusca of Australia. *Australian Zoologist,* 10: 188–230.

JEFFREYS, J. G., 1867, *The Mollusca.* Vol. IV. *Marine shells, British Conchology,* London, p. 1–486, pl. 1–4.

JOHANSSON, J., 1948, Über die Geschlechtsorgane der Hydrobiiden und Rissoiden und den ursprünglichen Hermaphroditismus der Prosobranchier. *Arkiv för Zoologi,* 40A(15): 1–13.

JOHANSSON, J., 1950, Über die weiblichen Geschlechtsorgane von *Hyala vitrea,* einer von dem *Rissoa*-Typus starck abweichenden Form der Gruppe Rissoacea. *Arkiv för Zoologi,* 42A(7): 1–6.

KOSUGE, S., 1965, Anatomical study on Japanese Rissoacea (I). On the family Rissoidae (I). *Venus, Japanese Journal of Malacology,* 24: 130–151, pl. 15.

KURODA, T. & HABE, T., 1950, Nomenclatural notes. *Illustrated Catalogue of Japanese Shells,* 2: 16.

KURODA, T. & HABE, T., 1954, New aquatic gastropods from Japan. *Venus, Japanese Journal of Malacology,* 18: 71–79.

LASERON, C. F., 1950, Review of the Rissoidae of New South Wales. *Records of the Australian Museum,* 22: 257–287.

LASERON, C. F., 1955, Revision of the New South Wales eulimoid shells. *Australian Zoologist,* 12: 83–107.

LASERON, C. F., 1956, The families Rissoinidae and Rissoidae (Mollusca) from the Solanderian and Dampierian Zoogeographical Provinces. *Australian Journal of Marine & Freshwater Research*, 7: 384–484.

LEA, I., 1833, *Contributions to Geology.* 227 p., 6 pl.

MARCUS, E. & MARCUS, E. du B.-R., 1963. Meso-

gastropoden von der Küste São Paulos. *Akademie der Wissenschaften und der Literatur, Abhandlungen der Mathematisch-Naturwissenschaftlichen Klasse,* 1: 1–105.

NEVILL, G., 1884, *Hand list of Mollusca in the Indian Museum, Calcutta. Part II. Gastropoda. Prosobranchia-Neurobranchia* (contd.). Government Printer, Calcutta. x + 306 p.

PALMER, K. v. W., 1937, The Claibornian Scaphopoda, Gastropoda and Dibranchiate Cephalopoda of the southern United States. *Bulletins of American Paleontology,* 7(32)1: 1–548; 2: 549–730. 90 pl.

PAVIA, G., 1975, I Molluschi del Pliocene inferiore di Monteu Roero (Alba, Italia NW). *Bollettino della Società Paleontologica Italiana,* 14: 99–175, 14 pl.

PILSBRY, H. A., 1922, Revision of W. M. Gabb's Tertiary Mollusca of Santo Domingo. *Proceedings of the Academy of Natural Sciences of Philadelphia,* 73: 305–435, pl. 16–47.

PONDER, W. F., 1967, The classification of the Rissoidae and Orbitestellidae with descriptions of some new taxa. *Transactions of the Royal Society of New Zealand, Zoology,* 9: 193–224.

PONDER, W. F., 1968, The morphology of some small New Zealand Prosobranchs. *Records of the Dominion Museum,* 6: 61–95.

PONDER, W. F., 1974, A review of the Australian species assigned to *Rissopsis* Garrett with a description of a new species of *Rissopsetia* (Mollusca: Gastropoda). *Journal of the Malacological Society of Australia,* 3: 25–35.

PONDER, W. F. & YOO, E. K., 1976, A revision of the Australian and tropical Indo-Pacific Tertiary and Recent species of *Pisinna (= Estea)* (Mollusca: Gastropoda: Rissoidae). *Records of the Australian Museum,* 30: 150–247.

RADOMAN, P., 1973, New classification of brackish water Prosobranchia from the Balkans and Asia Minor. *Prosebna Izdanja. Museum d'Histoire Naturelle de Beograd. Edition hors serie,* 32: 1–30.

SACCO, F., 1892, I molluschi dei terreni terziarii del Piemonte e della Liguria, parte 11. Eulimidae e Pyramidellidae (parte). *Memorie della R. Accademia delle Scienze di Torino,* ser. 2, 42: 1–100, 2 pl.

SEGUENZA, G., 1876, Studii stratigraphici sulla formazione pliocenica dell' Italia meridionale. *Bollettino del R. Comitato geologica d'Italia,* 7: 7–15, 91–103, 179–189, 260–271, 355–359 (part of series 1873–77).

STAROBOGATOV, Ya. I., 1970, Molluscan fauna and zoogeographical partitioning of continental water reservoirs of the world. *Akademiya Nauk SSSR. Zoologicheskii Institut Nauka, Leningrad,* 372 p. (in Russian).

STOLICZKA, F., 1867–1868, Cretaceous fauna of Southern India. 2. The Gastropoda. *Memoirs of the Geological Survey of India Palaeontologia indica,* Series V, 2, xiii + 500 p., 28 pl.

THIELE, J., 1928, Revision des Systems der Hydrobiiden und Melaniiden. *Zoologische Jahrbücher. Systematik, Ökologie und Geographie der Tiere.* 55: 351–402.

THIELE, J., 1929, *Handbuch der systematischen Weichtierkunde.* Jena. Part 1: 1–376.

THORSON, G., 1946, Reproduction and larval development of Danish marine bottom invertebrates. *Meddelelser fra Kommissionen for Danmarks Fiskeri- og Havundersogelser, serie: Plankton,* 4(1): 1–523, figs. 1–199.

WENZ, W., 1938–1944, Gastropoda. *Handbuch der Paläozoologie.* 6(1): Lief. 1–7. 1639 p. Berlin.

APPENDIX. DESCRIPTIONS OF NEW SPECIES OF IRAVADIIDAE

Genus *Iravadia*
Subgenus *Pseudonoba*
Iravadia (Pseudonoba) profundior Ponder, sp. nov.
Fig. 14G–I

Description. **Shell**. Small, elongately pupoid, rather solid, non-umbilicate. Protoconch small, smooth, glossy, of about two whorls, flat-topped, sharply-terminated. Teleconch of four convex whorls, sutures impressed. Fifteen spiral threads on penultimate whorl, 18 primary spirals on body whorl together with a few, weak secondary spiral threads and several weak spirals on basal fold. Axial sculpture of indistinct growth lines. Lower base with broad, rounded basal fold. Aperture rather large, oval, very weakly-excavated posteriorly, not angled, broadly and shallowly-excavated anteriorly. Outer lip slightly prosocline with sharp edge, thickened within, with broad, prominent varix. Inner lip thickened, narrow, attached to parietal area. Colour yellowish-white, periostracum yellow to brown, with ferruginous deposit in one of the two paratypes (Fig. 14G).

Dimensions.	length	diameter
Holotype	3.86 mm	1.47 mm
Paratype	4.24	1.51
	3.86	1.48

Operculum. Oval, columellar edge indented just beyond nucleus, which is displaced from middle of edge. A low, broad, internal fold parallel to columellar edge (Fig. 14H). **Radula**. Central teeth broadly-triangular, median cusp short, with several (six to nine) minute cusps on either side. A pair of weak denticles near lateral margins of central teeth. Lateral teeth with prominent primary cusp and

several (three to five) small cusps on outer side. Marginal teeth multicuspate (Fig. 14I).

Type-locality. Off Lusaran Light, Guimaras, Philippines, USBF Stn. 5183, in 96 fathoms (175 m), in soft mud.

Types. Holotype and two paratypes (USNM, 281302). All specimens collected alive.

Other material examined. Off Adyagan Is., E. Masbate, Philippines, USBF Stn. 5392, 135 fathoms (247 m): two shells (USNM, 281897).

Remarks. This new species is superficially similar to *Paronoba subquadrata* Laseron, 1950 (= *Iravadia (Pseudonoba) subquadrata* herein) in shell features but differs in being larger, in having a relatively thicker peristome with a much stronger varix, and in having stronger spiral sculpture. *Iravadia (Pseudonoba) bella* also has a similar shell but this differs in being larger, more inflated, with relatively weaker spiral sculpture and no basal fold.

The two additional specimens (USNM, 281897) referred to this species have smaller shells (maximum length 3.19 mm) than the type-series, the outer lip is orthocline, the aperture is weakly subangled posteriorly and the teleoconch consists of only 3½ whorls. They agree in most other details and consequently are regarded as a variety of *I. (P.) profundior.* Two lots in the USNM from Daram Channel, W. Samar, 32 fathoms (59 m) (USNM, 280751) and N of Marinduque, 50 fathoms (95 m) (USNM, 276116), have still smaller shells (maximum length 3.01 mm), with relatively stronger spiral sculpture and an orthocline to opisthocline outer lip. They are more similar to the two atypical specimens of *I. (P.) profoundior* (USNM, 281897) than to the type series and are not considered to be the same species. These specimens also have some similarity to *I. (P.) densilabrum.*

Iravadia (Pseudonoba) expansilabrum Ponder, sp. nov.
Fig. 16F–I

Description. **Shell**. Small, elongately-pupoid, thin, non-umbilicate. Protoconch small, smooth, glossy, with two whorls, flat-topped, sharply-terminated. Teleoconch of about 4¼ convex whorls, with moderately-impressed sutures. Sculpture of close, fine spiral threads (approximately 26 on penultimate whorl) and inconspicuous axial growth lines. Lower base with prominent fold sculptured with a few weak, spiral threads. Aperture oval, very weakly-subangled and excavated posteriorly; convex and broadly and shallowly excavated anteriorly. Outer lip slightly opisthocline, thickened within, with sharp edge, a thick varix behind. Inner lip broadly-expanded over parietal area as a thin callus, not expanded over basal fold. Colour yellowish-white, periostracum yellowish but with a thick, ferruginous coating in the two specimens collected alive (Fig. 16G).

Dimensions.	length	diameter
Holotype	3.18 mm	1.18 mm
Paratypes	2.95	1.10
	3.21	1.24
	3.03	1.14
	3.06	1.16

Operculum. Oval, columellar edge flattened, twisted outwards with a weak, internal, longitudinal fold parallel to it. Nucleus marginal, slightly displaced from middle of edge (Fig. 16H, I). **Radula**. Central teeth broadly-triangular, with strong cusps on narrow cutting edge $\frac{1 + 1 + 1}{1 \quad 1}$, a single pair of prominent denticles on face of each tooth. Lateral teeth with large primary cusps and possibly a small cusp on inside edge. Marginal teeth multicuspate (Fig. 16F).

Type-locality. c. 40 miles (64 km) WSW off Tulear, Malagasy Republic, 23°19′S, 43°36′E, 82 m. 6 August, 1964. A. Bruun Stn. 363W.

Types. Holotype and five paratypes (USNM, 717549). Holotype coated with gold.

Remarks. This species is closest to *I. (P.) subquadrata* but its shell differs from that species and all others in the genus by its broadly expanded inner lip. The radular features, especially those of the central teeth, are unusual for *Iravadia,* and are discussed in the remarks on *Pseudonoba* above.

Iravadia (Pseudonoba) gemmata Ponder, sp. nov.
Fig. 16A–E

Description. **Shell**. Small, very elongately-conic, solid, non-umbilicate. Protoconch small, smooth, glossy, of 2–2½ whorls, flat-topped, sharply-terminated (Fig. 16B). Teleoconch of about six convex whorls, with deeply-impressed sutures. Sculpture of about 17 nodulous spiral ribs on body whorl, 12–14

on penultimate whorl, crossed by weak, close, prosocline axial riblets with small, rounded gemmules at points of intersection. Lower base with prominent, sharp fold, smooth or with weak spiral striae, partly overlaid by inner lip. Aperture rather large, oval, with weak angulation and excavation posteriorly, shallowly and broadly excavated anteriorly; inner lip broad, thickened, attached to parietal area, its outer edge convex; outer lip thickened at edge and behind, slightly prosocline, with a wide, flat-topped varix immediately behind. Colour white, periostracum yellow to brown, thin. Some specimens coated with a ferruginous deposit which stains shell orange-brown to nearly black (Fig. 16A).

Dimensions.	length	diameter
Holotype	5.52 mm	1.85 mm
Paratypes (277683)	5.33	1.78
	4.95	1.62
	5.27	1.80
	5.12	1.65
(283016)	4.73	1.65

Operculum. Oval, columellar edge convex, nucleus near middle of edge. No internal ridges (Fig. 16C). **Radula**. Central teeth broad, dorsal margin convex, cutting edge narrow, with one or two weak lateral cusps and a long, sharp median cusp; lateral margins widely flared, thickened. Three pairs of strong, closely-spaced denticles arise from face of tooth just below lateral margin; fourth pair of weak denticles below outer part of lateral margin. Middle of face of central tooth with a vertical, thickened band; ventral edge of tooth convex. Lateral teeth elongate, with a single, long, sharp cusp at inner edge and very weak cusp outside this cusp; otherwise simple. Marginal teeth with numerous sharp cusps (Fig. 16D, E).

Type-locality. Off Limbancauayan Is., W. Samar, Philippines; 50 fathoms (91.4 m), fine, grey sand, USBF Stn. 5210.

Types. Holotype (USNM, 283016a) and three paratypes (two juveniles) (USNM, 283016b); 16 paratypes (one juvenile) (USNM, 177683). Holotype coated with gold.

Other material examined. Buton Strait, Celebes (Sulawesi), 37 fathoms (68 m), USBF Stn. 5642: ten specimens (USNM, 279749), one specimen (USNM, 290829). Philippines: S of Corregidor Lt., 35 fathoms (64 m), USBF Stn. 5100: two specimens (USNM, 257398), two specimens (USNM 257397). North off Marinduque, 50 fathoms (91 m), USBF Stn. 5220: 17 specimens (USNM, 276115). North off Marinduque, 193 fathoms (353 m), USBF Stn. 5221: one specimen (USNM, 277065). Lagonoy, East Luzon, 47 fathoms (86 m), USBF Stn. 5448: one specimen (USNM, 289018). Off Tacbuc Point, East Leyte, 62 fathoms (113 m), USBF Stn. 5479: four specimens (USNM, 283016). Off Magabao Is., East Mindanao, 494 fathoms (903 m), USBF Stn. 5236: one specimen (USNM, 276875).

Remarks. The radula of this species differs from all others known in the genus by the characters of the central teeth, as discussed in the remarks on the subgenus *Pseudonoba* above. The shell is readily separated from other species of *Pseudonoba* by its gemmate sculpture. The operculum is closest to that of *Iravadia (Pseudomerelina) mahimensis* in morphology.

This species probably represents a separate grouping within, or close to, *Iravadia* s.l. but, until the radulae of more species of *Iravadia (Pseudonoba*) are known, allowing its relationships to be more accurately assessed, it can be tentatively retained in *Pseudonoba.*

The denticles on the face of the central teeth appear to have increased in size and number to take over the scraping function of the lateral cusps, the original cutting edge being reduced in size. Species of *Iravadia,* typically have several small cusps and weaker (i.e., less thickened) teeth.

Genus *Chevallieria*

Chevallieria australis Ponder, sp. nov.
Fig. 17D–F

Description. **Shell**. Minute, pupoid, thin, non-umbilicate. Protoconch relatively large, of 1¾ whorls, flat on top, terminated abruptly, smooth. Teleoconch of about 2⅓ convex whorls, sutures lightly impressed. Surface smooth except for indistinct axial growth lines. Aperture rather large, oval, subangled and very slightly channelled posteriorly, broadly and very shallowly excavated anteriorly. Outer lip orthocline, slightly thickened within, no external varix. Inner lip thickened, narrow upper half attached to, or free from, parietal wall, lower half free. Periostracum thin, yellowish-brown (Fig. 17D).

Dimensions.	length	diameter
Holotype	1.50 mm	0.68 mm
Paratype	1.64	0.75

Operculum. (Fig. 17E) and **radula** (Fig. 17F) as described under generic diagnosis for *Chevallieria*.

Type-locality. Emu Bay, N.E. Kangaroo Is., South Australia, on sheltered, rocky shore under rocks at low water neap; 7 March 1978. Collected by I. Loch, E. K. Yoo and K. Handley.

Types. Holotype (C.126181) and paratype (C.126182), AMS.

Other material examined. Point Sinclair, S. Australia, two shells (AMS, C.126183); Largs Bay, S. Australia, one shell (SAM); Tumby Bay, S. Australia, one shell (AMS, C.126184); Bathurst Point, Rottnest Is., Western Australia, one shell (AMS, C.126185).

Remarks. This species is closest to *Chevallieria columen* (Melvill) but the shell of that species is larger (greater than 2.5 mm in length), relatively narrower and the teleoconch consists of about 3½ whorls. The new species and *C. columen* differ from the other Recent species described from Australia, *C. imitoris* (Laseron), in lacking fine spiral striae on the surface of the shell. *Chevallieria imitoris* also has a relatively narrower shell than *C. australis*. It is known from Darwin, N Australia (the type-locality) and Queensland. Other specimens of *Chevallieria* from the tropical Indo-Pacific appear to include unnamed species, but insufficient material is available to describe these.

Chevallieria balcombensis Ponder, sp. nov.
Fig. 17B

Description. **Shell**. Minute, pupoid, thin, non-umbilicate. Protoconch relatively large, of two whorls, flat on top, terminated abruptly. Teleoconch of about three convex whorls, sutures slightly impressed. Surface smooth, shining, with weak axial growth lines the only sculpture. Aperture rather large, oval, weakly-subangled and very shallowly channelled posteriorly, broadly and shallowly-channelled anteriorly. Outer lip very slightly opisthocline to very slightly prosocline, thickened slightly within, with sharp edge and prominent, rounded external varix. Inner lip narrow, thickened, attached or partially free from parietal wall (Fig. 17B).

Dimensions.	length	diameter
Holotype	1.98 mm	0.79 mm
Paratypes	2.24	0.83
	1.98	0.79
	1.62	0.73
	1.90	0.79
Bird Rock Cliffs	2.20	0.92

Type-localty. Fossil Beach, Balcombe Bay, Mornington, Victoria, Australia. Middle Miocene (Balcombian), J. Voorwinde Colln.

Types. Holotype (C.126186) and eight paratypes (C.126187), AMS; two paratypes NMV, P.62046.

Other material examined. Topotypes, W. J. Parr Colln., two specimens (NMV, P.62044, 62047); Bird Rock Cliffs, Torquay, Victoria, Lower Miocene-Upper Oligocene (Janjukian) one specimen (NMV, P.62045).

Remarks. Of the Australian species, the shell of *C. balcombensis* is closest to *C. australis* in lacking spiral striae, but it differs in its slightly larger size and prominent apertural varix.

Chevallieria gippslandica Ponder, sp. nov.
Fig. 17C

Description. **Shell**. Small, elongately-conical, rather solid, non-umbilicate. Protoconch small, smooth, of two whorls, flat-topped, sharply-terminated. Teleoconch of 4¼ convex whorls; sutures impressed. Sculpture of weak, axial growth lines only. Aperture oval, subangled and with shallow channel posteriorly, broadly and slightly excavated anteriorly. Outer lip slightly opisthocline, thickened within, with a sharp edge and flat, weak to prominent external varix. Inner lip narrow, attached to, or separated from, parietal wall (Fig. 17C).

Dimensions.	length	diameter
Holotype	3.82 mm	1.44 mm
Paratypes	3.58	1.41
	3.57	1.32
	3.18	1.18

Type-locality. Roadcutting, right bank of Meringa Creek, 100 m S of Kalimna-Nungerner Road, Gippsland, E. Victoria, Australia. In bed below Lower Jemmys Point shell bed, Jemmys Point Formation, Kalimnan, Lower Pliocene. Collected by W. F. Ponder, T. A. Darragh and P. H. Colman, 11 Jan. 1970.

Types. Holotype (C.126207) and three paratypes (C. 126208), AMS; two paratypes, NMV, P.62048 from type-locality, collected Dec. 1966.

Other material examined. Kalimnan: Ditch on E side of Meringa Creek, in lowest shell band four m above creek (NMV, P.62048, two paratypes); SW side of Bunga Creek, road cutting Princes Highway, Gippsland, E. Victoria, one specimen (NMV, P.62049); zero to two m above beach in cliff 50–100 m E of Kalimna Jetty, in lower shell bed, Gippsland, E. Victoria (NMV, P.62051). Cheltenhamian: NE side Bunga Creek, road cutting, Princes Highway, Gippsland, E. Victoria; one specimen (NMV, P.62050).

Remarks. This species is relatively large for the genus and resembles species of *Iravadia (Pseudonoba)* in general shell characters. It lacks, however, any trace of spiral sculpture and does not have a basal fold. It appears to be derived from *Chevallieria balcombensis*, which it closely resembles, except in size and number of whorls.

Genus *Nozeba*

Nozeba (?) striata Ponder, sp. nov.
Fig. 20G–J

Description. **Shell.** Small, elongately-ovate, rather thin, with dull surface. Protoconch smooth, of moderate size, of 1¾ whorls, nucleus very small, depressed. Teleoconch of 2½ convex whorls, sutures impressed. Sculpture of fine, close, raised spiral lines with linear interspaces and weak axial growth lines. Aperture sub-oval with broad anterior notch and posterior subangulation. Outer lip orthocline, lacking external varix; inner lip narrow, slightly thickened, free in anterior half. Umbilical chink small in holotype, more pronounced in paratype (Fig. 20G).

Dimensions.	length	diameter
Holotype	1.84 mm	1.05 mm
Paratype	1.69	1.03

Operculum. Thin, simple, spiral, nucleus eccentric (Fig. 20J). **Radula**. Central teeth broad, cutting edge concave, lateral wings only slightly thickened, at about 45° to vertical; median cusp very small, blunt, six to seven lateral cusps on each side, narrow, sharp, inner ones about twice length of median cusp; no basal denticles, lateral margins slightly thickened. Lateral teeth with narrow cutting edge, primary cusp moderately long, sharp, secondary cusps sharp, c. 3 + 1 + c. 6. Marginal teeth with broad, almost straight cutting edges and long bases, with numerous small, sharp cusps almost equal in length to secondary cusps on lateral and central teeth, but narrower (Fig. 20H, I).

Type-locality. Pujada Bay, E Mindanao, Philippines, 218 fathoms (399 m) USBF Stn. 5243.

Types. Holotype and paratype (USNM, 311059). Holotype coated with gold.

Remarks. This species differs from the two Australasian species of *Nozeba*, for which the radula is known, in not having a pair of basal denticles on the central tooth of the radula, and in the median cusp of the central teeth being relatively very small. The shell, however, has all the essential characters of *Nozeba* species as diagnosed herein, except that it lacks a glossy surface. It differs from the described species by the combination of the following characters: the relatively large aperture, the spiral sculpture, the dull surface, the rather solid shell and the umbilical chink. Only two other described species have distinct spiral sculpture: *N. emarginata* and *N. gatliffiana*.

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POTAMOLITHUS: MORPHOLOGY, CONVERGENCE, AND RELATIONSHIPS AMONG HYDROBIOID SNAILS

George M. Davis[1,2] & Maria Cristina Pons da Silva[1,3]

ABSTRACT

Heuristic evaluations of the origin, evolution and deployment of faunas depend on thorough systematic studies. Systematic relationships among rissoacean taxa cannot be adequately assessed without comparative anatomy of all organ systems and ontogenetic analyses of some of them (Davis, 1979). The cohesiveness of the morphological groundplan seen in an endemic adaptive radiation may assure the anatomist that the taxa of that radiation are monophyletic. To state that geographically separated radiations are monophyletic and to show their relationships cladistically is more complex. The problems and constraints have been discussed lucidly (Cain & Harrison, 1960; Cain, 1964). The major problems are convergence and convincing assessments of homologies.

The Hydrobiidae *sensu lato* were defined by similarities of the shell, radula, operculum, and penis. The inadequacy of describing higher taxa by such characters has already been shown (Davis, 1979, 1980). At least 30% to 40% of the characters used to discriminate rissoacean taxa are from the female reproductive system. Shell characters are the least reliable because of convergence (Davis, 1979, 1980, 1981).

In the late nineteenth century snails in various places of the world were placed in *Lithoglyphus* because of a globose to cap-shaped shell, simple penis, and presence of three or four pairs of basal cusps on the rachidian radular tooth. *Lithoglyphus s.s.* is European and the type-species, *L. naticoides* Pfeiffer, is the standard for the hydrobiid subfamily Lithoglyphinae (Davis *et al.*, 1976, Appendix). Species of so-called *Lithoglyphus* of western Yunnan, China are in the Pomatiopsidae: Triculinae (Davis, 1979). *Lithoglyphus* from southern Brasil (von Ihering, 1885) and Uruguay was monographed by Pilsbry (1911), who placed some thirty species in a new genus *Potamolithus* in the Amnicolidae (= Hydrobiidae). While several species of *Potamolithus* clearly resemble triculine *Lacunopsis* of the Mekong River, an analysis of the female reproductive system of *Potamolithus* was needed to confirm the suspected relationship. The possibility existed that *Potamolithus* converges on taxa of the Triculinae because it lives on rocks in high energy environments (Davis, 1979).

Anatomical data for *Potamolithus ribeirensis* Pilsbry show that this species is not a pomatiopsid but a hydrobiid with closest relationships to *Lithoglyphus s.s.* Some differences clearly show divergence between these genera while other differences may or may not be shown to be real, pending a more detailed analysis of *Lithoglyphus s.s.* These findings tend to negate a Gondwanaland origin for *Potamolithus*. As no Triculinae are found in South Africa (Davis, 1981) and *Potamolithus* is not a triculine, the Triculinae more probably originated on the Indian Plate section on Gondwanaland. *Potamolithus* may be closely related to North American genera such as *Somatogyrus* and *Fluminicola*, as evidenced by similar shells, radulae, opercula, and penises. However, with convergence in mind, evidence for this must come from detailed anatomical studies of these North American genera.

Key words: *Potamolithus*; Hydrobiidae; morphology; systematics; convergent evolution; South America; Brasil.

INTRODUCTION

There is considerable current interest in coupling phylogenetic and zoogeographic analyses to understand the faunistic patterns seen in the world today. There are serious constraints in assessing phylogeny and in many cases it may not be possible to do so (Cain & Harrison, 1960; Cain, 1964). Major problems include lack of sufficient characters and qualitatively different character-states, convergence, and a correct evaluation of

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homologies. It is also important to realize that faunistic spatial patterns result from the sum of vicariant events, dispersal events, and variance of ecological factors through time (Davis, 1981).

Adaptive radiations in different parts of the world and at different times, in which the species resemble each other phenetically, are of considerable interest to the evolutionary biologist and zoogeographer. Do the resemblances reflect phylogenetic relationships or convergences?

In attempting to understand something of the origin and evolution of the rissoacean family Pomatiopsidae, it was noted that various species from around the world were placed in the genus *Lithoglyphus* by early authors (Davis, 1979). Species were placed in *Lithoglyphus* because of the resemblances in their shells and opercula (as well as radulae and penises in some cases) to those in *Lithoglyphus naticoides*, the type-species. These features are a large globose shell (4 to 6 mm length), two or more pairs of basal cusps on the central tooth; one or more pairs arise from the face of the tooth, not the lateral angle. Penis is without appendages. The operculum is corneous and paucispiral. It was shown that *Lithoglyphus s.s.* of Europe converges on species of so-called *Lithoglyphus* and other similar looking species of *Lacunopsis* from the Yangtze and Mekong rivers of Southeast Asia (Davis, 1979). European *Lithoglyphus* belongs in Hydrobiidae: Lithoglyphinae while the Asian and Southeast Asian taxa in Pomatiopsidae: Triculinae. *Lithoglyphus* of Lake Tanganyika, Africa, later considered *Lacunopsis*, is now known as *Spekia*, an endemic monospecific cerithiacean genus that converges on *Lacunopsis harmandi* of the Mekong River (Davis, 1979).

The head and penis of *Lithoglyphus lapi-*

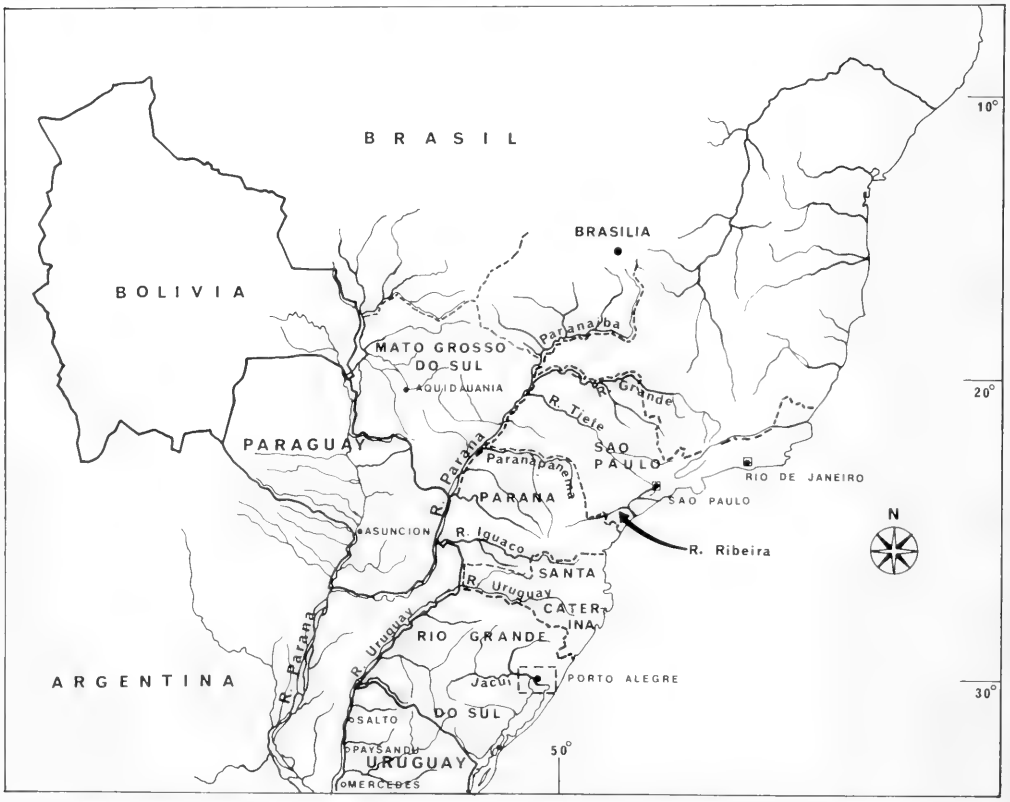

FIG. 1. Map showing the most important localities for *Potamolithus* along the Uruguay and Parana rivers. Paysandu in Uruguay is the type-locality of many species of *Potamolithus*. The type-locality of *P. ribeirensis* is shown by the arrow. Our study area is shown by the box of dashed lines around Porto Alegre. The region where the Pomatiopsidae: Pomatiopsinae *Aquidauania* comes from is shown in Mato Grosso do Sul State of Brasil.

dum Orbigny from southern Brasil were illustrated by von Ihering (1885). The animal, shell and radula so much resembled certain species of *Lacunopsis* (a genus endemic to the Mekong River) that the question arose as to whether South American *Lithoglyphus* was in the Triculinae or phenetically related to the Triculinae; or was the resemblance due to convergence caused by life on rocks and stones in a fluviatile environment (Davis, 1979, pp. 30, 33)? Pilsbry (1896, 1911) created the genus *Potamolithus* for the South American radiation that includes *L. lapidum*. The type-species is *P. rushii* Pilsbry, 1896 by original designation. The *Potamolithus* radiation comprises some thirty species that particularly abound in the Plata-Uruguay River drainages.

We present the anatomy of *P. ribeirensis* to answer questions raised about phylogenetic relationships and convergence. *P. ribeirensis* is a species discussed by von Ihering (1885) as *Lithoglyphus lapidum*. We show that *Potamolithus* converges on certain Triculinae and is a member of the Hydrobiidae: Lithoglyphinae if we have correctly interpreted the published anatomy of *Lithoglyphus naticoides* (Krull, 1935; Krause, 1939; Radoman, 1966). This study underscores once again, that convergence must not be underestimated when attempting phylogenetic and zoogeographic analyses.

MATERIALS AND METHODS

Localities

Feitoria River, 100–200 m upstream from bridge crossing on federal road BR116 from Novo Hamburgo to Dois Irmaos, Rio Grande do Sul, Brasil; 29°35′S, 51°07′W; 19, 21 March 1981 (Figs. 1, 2). Academy of Natural Sciences (ANSP) catalog nos.: 353484; A8727; 353485; A8728.

Type-locality for *Potamolithus ribeirensis*

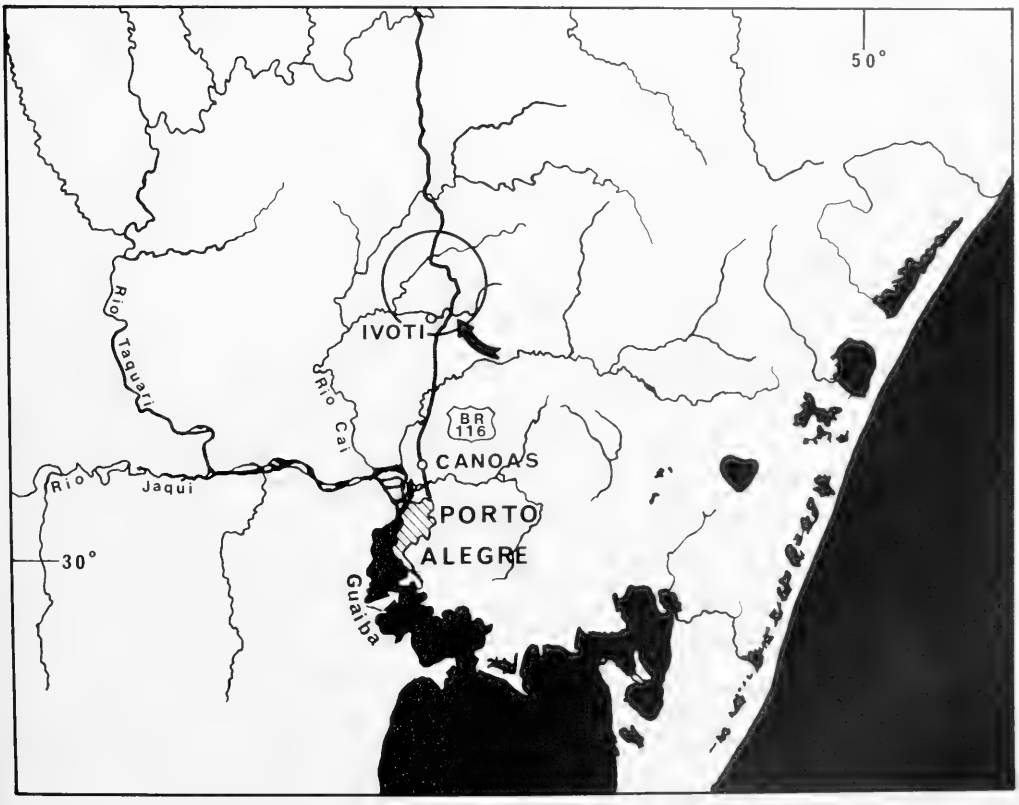

FIG. 2. Map showing details of our collecting site for *Potamolithus ribeirensis* N of Porto Alegre, Brasil. The arrow marks the locality.

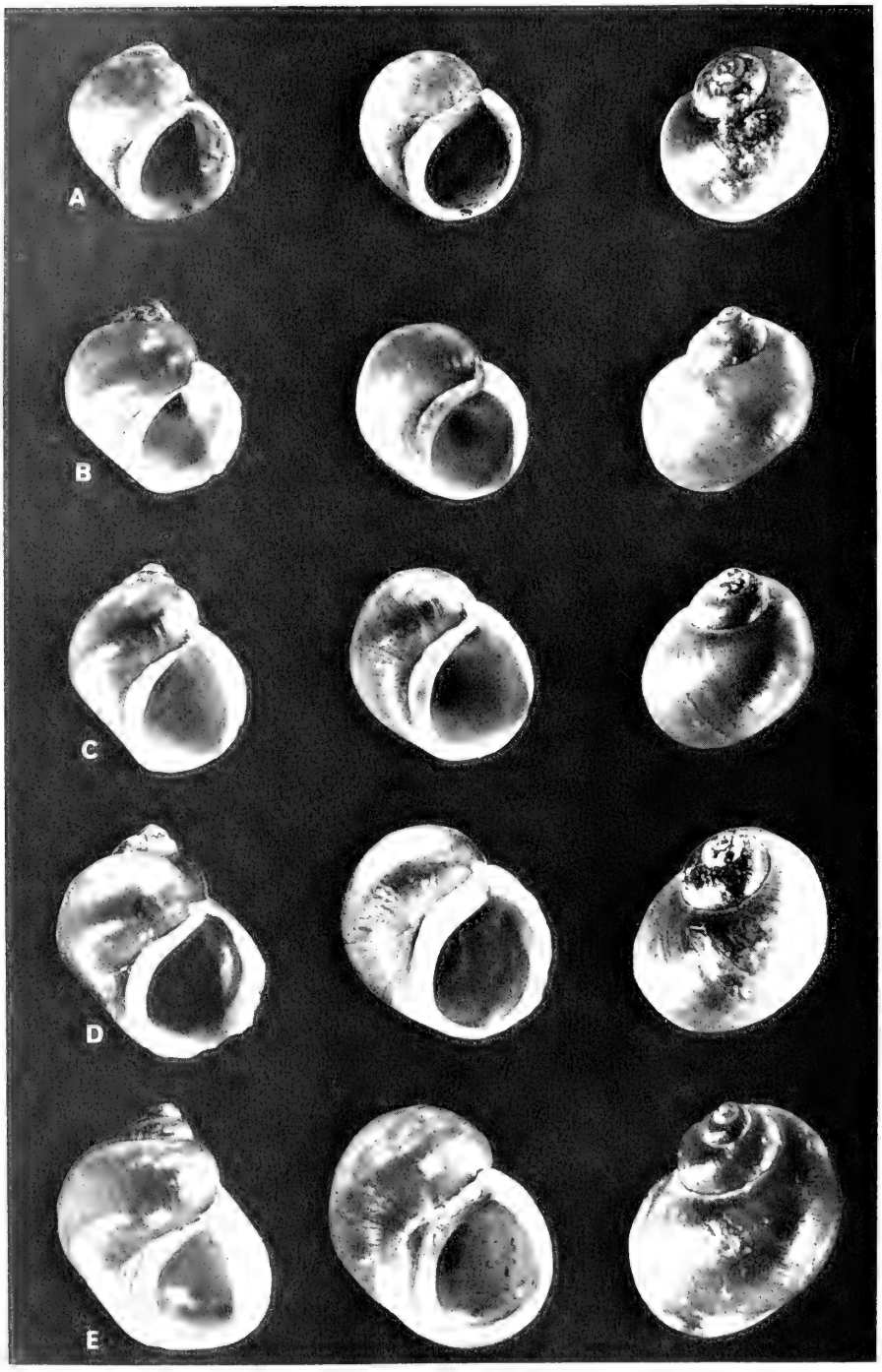

FIG. 3. Shells of *Potamolithus ribeirensis*. A. Lectotype, ANSP 103076. Shell length is 3.68 mm. Other shells printed at the same magnification. Shells B–E are from our study site (ANSP 353484). Shell E has a pronounced keel and excavation of the umbilical region.

Pilsbry. Rio Ribeira, Yporanga (or Iporanga), São Paulo, Brasil; 24°35′S, 48°35′W. Lectotype, ANSP 103076 (Fig. 3A). See Appendix.

Habitat

Mountain stream of clear, clean water flowing over stones and boulders; intermittent pools with sandy bottom. *P. ribeirensis* crowded on all sides of stones and boulders at stream margin where there was continuous current. Snails absent, however, in swift current and white-water rapids. Associated fauna: *Chilina* sp. (Chilinidae) in microsympatry with *P. ribeirensis*, but fewer in numbers, approximately 500 to 1. In depositional quiet-water areas, *Littoridina* sp. sparse; some *Pisidium* (Pisidiidae) also.

Collection and methods

Methods are those presented in detail by Davis & Carney (1973), Davis *et al.* (1976), and Davis (1979). Characters that are shared with other rissoaceans or that are standard in the Hydrobiidae and Pomatiopsidae are not discussed here. Examples of such characters are the style sac, fecal pellets, structure of the ctenidium, etc. Statistical analyses are 1) the standard "t" test for congruency of means, 2) X^2 analysis, and 3) multivariate morphometric analyses of shell parameters using NT-SYS (Rohlf *et al.,* 1972) with details given in the Appendix.

We took a random sample of the population to determine whether there was sexual dimorphism. The sample consisted of 184 individuals drawn from an initial population of over 1000 individuals, which were all the individuals of all size classes at a single site in the river. We determined the number of individuals and their sex for each whorl number. We determined the shell length and length of body whorl for males and females of 4.0 and 4.5 whorls.

Multivariate analysis

The data matrix consisted of 8 OTUs and 32 characters. *Hydrobia truncata* represents the genus *Hydrobia* as typical of the Hydrobiidae: Hydrobiinae. All species of *Lacunopsis* have the character states as scored. *Lacunopsis* is the sole genus of the tribe Lacunopsini of the Pomatiopsidae: Triculinae (Davis, 1979). *Pomatiopsis lapidaria* and *Tomichia ventricosa* are representatives of the Pomatiopsidae: Pomatiopsinae. *Tricula aperta* and *T. bollingi* are representatives of the generalized Pomatiopsidae: Triculinae: Triculini. A more comprehensive cladistic analysis of genera and species of the Pomatiopsidae: Triculinae: Triculini has been presented elsewhere (Davis & Greer, 1980). *Lithoglyphus naticoides* represents the Hydrobiidae: Lithoglyphinae.

Of the characters used, 3 (9%) are from the shell; 7 (22%) involve head-foot-mantle; 1 (3%) mantle cavity; 2 (6%) radula; 5 (16%) male reproductive system; 11 (34%) female reproductive system; 3 (9%) involve one each for stomach, nervous system, and eggs.

Computations were made using the June 1974 version of NT-SYS (Rohlf *et al.,* 1972). Q and R mode analyses were performed as in Hoagland & Davis (1979). Non-metric multidimensional scaling (MDS) was emphasized. Limited reliance can be placed on cluster analyses and phenograms to illustrate relationships. Ordination and MDS are freed from the constraints of phenogram construction. Ordination diagrams based on three-dimensional scaling are presented with subsets. The subsets algorithm groups OTUs so that the largest distance between OTUs within a group is less than the distance between any group member and any candidate for membership.

We present a table for character loading on each principal component. We discuss character correlations as pertinent to later discussions of relationships among taxa and problems caused by convergence.

Cladistic analysis

We present a cladistic analysis based on a set-theory solution involving unique and unreversed characters *sensu* Wilson (1965), and Davis & Greer (1980). The cladistic and multivariate analyses complement each other in our assessment of relationships.

RESULTS: MORPHOLOGY

Shells of the Feitoria population

Shells of the Feitoria population are globose, imperforate, and solid, but have a weak outer lip (Figs. 3, 4). Shells with heavy periostracum. The spire is low; there are 4.0 to 4.5 whorls. The body whorl is regularly convex in most shells but slightly flattened in a few. The aperture is oblique, inclined about

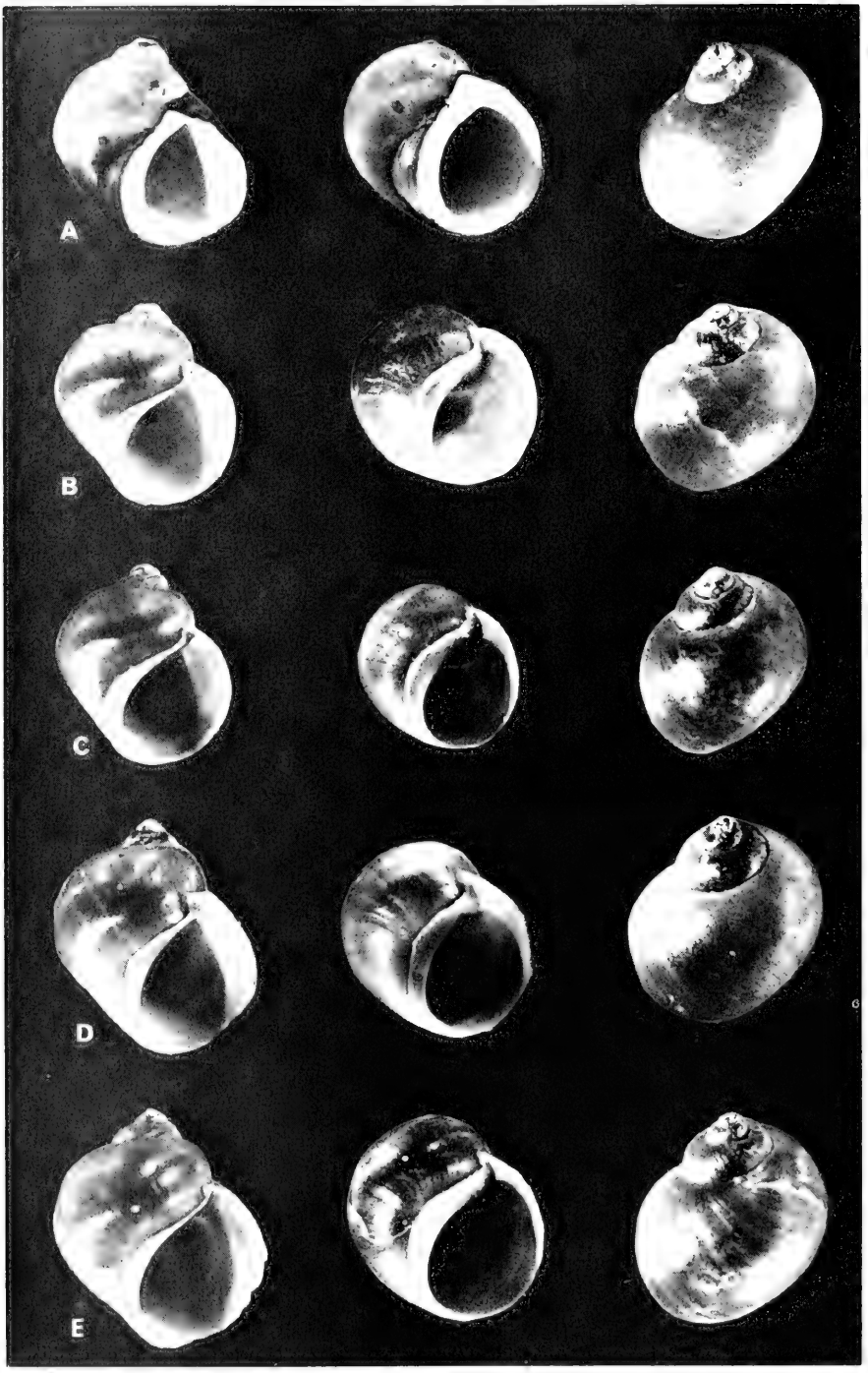

FIG. 4. Shells of *Potamolithus ribeirensis*. Shell A was figured by Pilsbry (see Appendix). ANSP 103068; length is 4.04 mm. Other shells are printed at the same magnification. Shells B–E are from our study site showing variation in shape.

55° to 40° toward the axis of coiling. The aperture flares at the adapical end, rounded to angular where the columella meets the outer lip. The columella is heavy and calloused; it is wide, flat or slightly concave. The parietal callus is weak at the edge, so that the peristome is not complete or barely complete in 95% of all individuals; it is heavy within. A thick, complete parietal callus is only seen on old individuals.

The umbilical area is extremely narrow. In some individuals a sharp keel circles from the umbilical area to the adapical end of the inner lip (Figs. 3D, E). In older individuals the umbilical area can be eroded and excavated to expose an umbilical opening.

The Feitoria population mostly consisted of males and females of 4.0 whorls (72.6%; Table 1). Only 8.6% of the population attained 4.5 whorls. Individuals of 3.5 whorls and larger were sexually mature. There was no significant difference in numbers of males and females (Table 1). Shell dimensions are given in Tables 2–4. There was no sexual dimorphism in shell size (Table 2).

Most snails had 4.0 whorls. Size-frequency histograms of males and females with 4.0 whorls show a normal distribution if both males and females are considered together (Fig. 5). However, considering males and females separately, in the center of the distribution (3.2 to 3.4 mm) there is a deficiency of males. A X^2 analysis of numbers in this size class versus numbers in all other size classes combined suggests a significant difference between sexes ($P < .10$) in this medium class.

Shell dimensions of the syntypic series are given in Table 3. The syntypes only attained 3.5 whorls; the parietal callus is more pronounced in the specimens compared with the condition seen in Feitoria specimens. Shell morphometric analyses of individuals from the two populations are given in the Appendix.

Shell dimensions of those individuals used for the anatomical analyses are given in Table 4.

TABLE 1. Random sample of 186 individuals from a universe of >1000 individuals, showing the frequencies of males and females at each whorl stage. N = 186.

Whorls	♂	♀	% of population
eroded	0	2	1.1
3.0	2	3	2.7
3.5	11	17	15.1
4.0	66	69	72.6
4.5	7	9	8.6
N =	86	100	

Head-foot

The head is broad and squat with thick stubby tentacles (Fig. 6). The snout is relatively short and narrow compared to the width of the neck. The foot is wide and powerful, with the usual anterior mucous groove. There is no pronounced omniphoric groove, suprapedal fold, or pedal crease, as in the Pomatiopsinae. The operculum is corneous, paucispiral, with the position of the nucleus as figured for *Somatogyrus* or *Fluminicola* by

TABLE 2. Shell length (L) and length of body whorl (LBW) (mm) for males and females of 4.0 and 4.5 whorls. N = number measured; $\bar{X} \pm$ Sd; (range). SD = significant difference between males and females; $\bar{X}$ = mean; Sd = standard deviation.

		♂	♀	SD $P \leq .10$
L	4.0	3.41 ± 0.44 (2.40 – 4.32) N = 50	3.46 ± 0.43 (2.48 – 4.56) N = 50	No
	4.5	4.09 ± 0.44 (3.28 – 4.72) N = 7	4.31 ± 0.24 (3.88 – 4.52) N = 9	No
LBW	4.0	2.98 ± 0.48 (2.00 – 3.96) N = 50	3.02 ± 0.40 (2.32 – 3.76) N = 50	No
	4.5	3.64 ± 0.46 (2.72 – 4.12) N = 7	3.85 ± 0.18 (3.52 – 4.44) N = 7	No

TABLE 3. A comparison of shell measurements of the type-series and specimens from the Feitoria population.

	Type-locality: race-A								
	No.	Whorl no.	Length	Width	Length of body whorl	Length of aperture	Width of aperture	Width of penultimate whorl	Width of columellar callus
Lectotype ANSP	1	3.5	3.68	3.36	3.40	2.92	2.44	1.0	0.48
Paralecto-types ANSP	5	3.0 to 3.5	3.15 ± 0.59 (2.28 – 3.68)	2.90 ± 0.45 (2.28 – 3.40)	2.82 ± 0.53 (2.04 – 3.32)	2.49 ± 0.43 (1.88 – 2.96)	2.10 ± 0.36 (1.6 – 2.48)	0.88 ± 0.18 (0.64 – 1.04)	0.49 ± 0.09 (0.36 – 0.60)
	Feitoria River: race-B								
	17	3.5	2.45 ± 0.45 (1.84 – 3.24)	—	2.09 ± 0.41 (1.56 – 2.84)	—	—	—	—
	6	4.0	3.63 ± 0.18 (3.4 – 3.92)	3.22 ± 0.23 (2.96 – 3.36)	3.22 ± 0.19 (2.96 – 3.52)	2.81 ± 0.20 (2.56 – 3.16)	2.33 ± 0.17 (2.2 – 2.6)	1.07 ± 0.05 (1.0 – 1.12)	0.46 ± 0.04 (0.4 – 0.52)
	4	4.5	4.65 ± 0.26 (4.60 – 5.00)	4.04 ± 0.15 (3.88 – 4.24)	4.07 ± 0.23 (3.8 – 4.4)	3.29 ± 0.15 (3.12 – 3.44)	3.05 ± 0.13 (2.96 – 3.24)	1.3 ± 0.10 (1.2 – 1.44)	0.48 ± 0.03 (0.44 – 0.52)

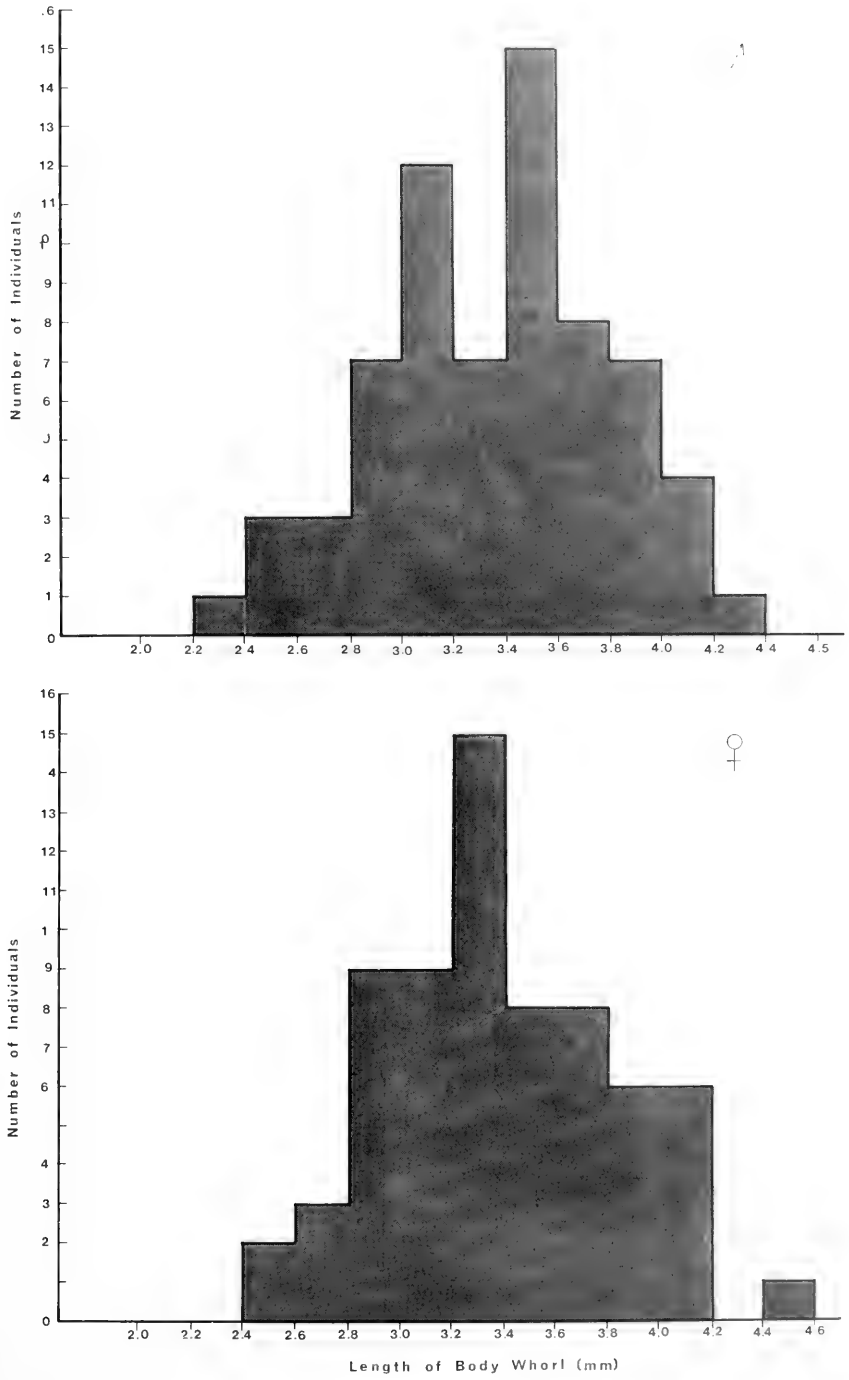

FIG. 5. Histogram of snail sizes (males and females) with four whorls, based on length of the body whorl. From a random sample.

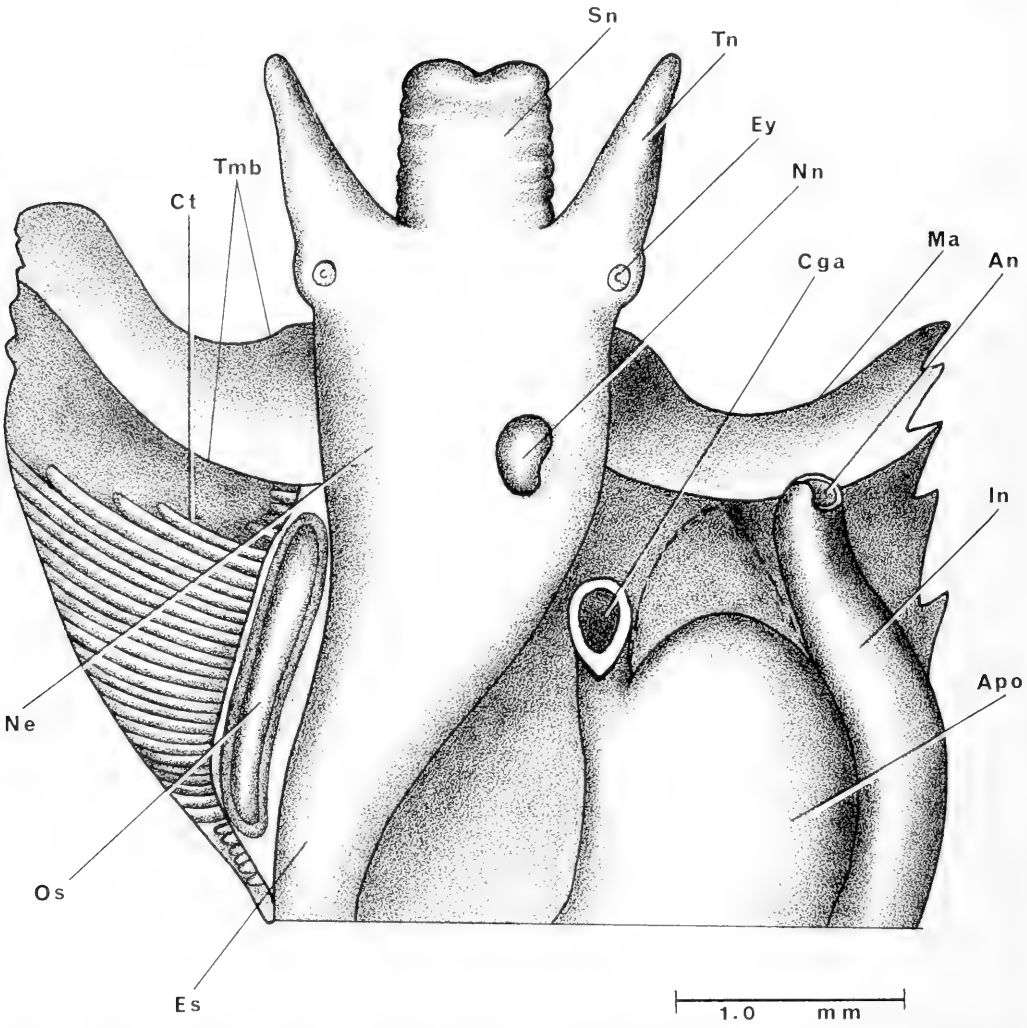

FIG. 6. Head and mantle cavity of a female. Note the 1) elongate osphradium, 2) thickened wide mantle border (Tmb), 3) stubby tentacles (TN), 4) nuchal node (Nn), and 5) position and prominence of the common genital aperture (Cga). An, anus; Apo, anterior pallial oviduct = capsule gland; Cga, common genital aperture; Ct, ctenidium; Es, esophagus; Ey, eye; In, intestine; Ma, edge of the mantle; Ne, neck; Nn, nuchal node; Os, osphradium; Sn, snout; Tmb, thick, wide mantle border; Tn, tentacle.

Walker (1918). The entire head is black with melanin.

On the neck of females, to the right of the mid-line, is a pronounced, fleshy protuberance here called the nuchal node (Nn, Fig. 6). It is situated where the base of the penis is located in males.

Mantle cavity

The mantle cavity is the standard hydrobioid type. Organ dimensions are given in Table 5. Three features are distinctive. The mantle collar (Ma, Fig. 6) has a very wide and thickened mantle border (Tmb). The osphradium is extremely long, 74 to 76% the length of the ctenidium's base. The osphradium is set back from the anteriormost part of the mantle cavity because it does not overlap the wide mantle border (Tmb). In the female, the common genital opening (Cga) opens upward from the floor of the mantle cavity next to the neck, as a wide, prominent slit. There is no sexual dimorphism in number of gill filaments;

they average 26.5 and 27.8 for males and females respectively (Table 5).

Female reproductive system

The uncoiled female without head and with kidney tissue removed is shown in Fig. 7. Organ dimensions are given in Table 5. The distinctive features are: 1) The pallial oviduct complex is squeezed over along the columellar muscle (in contrast to the usual position along the left ventrolateral aspect usually seen—Fig. 6, Davis & Greer, 1980, Pomatiopsidae: Triculinae); 2) the anterior end of the pallial oviduct (Apo) is far removed from the mantle collar (Ma); 3) the digestive gland (Di) covers the posterior part of the stomach (in contrast to being posterior to the stomach as in the Pomatiopsidae). The gonad is the generalized type (Fig. 11, Davis, 1980).

The interrelationships of the bursa copulatrix (Bu), seminal receptacle (Sr) and pallial oviduct (Ppo and Apo) are shown in Fig. 8. The orientation of these organs is the same shown in Fig. 7 except that, as the arrow

TABLE 4. Shell measurements (mm) for five males and females of 4.0 to 4.5 whorls from which organ measurements were made. $\bar{X} \pm$ standard deviation (range).

	♂	♀
Length	4.26 ± 0.24 (3.92 – 4.48)	4.63 ± 0.44 (4.04 – 5.00)
Width	3.69 ± 0.17 (3.44 – 3.88)	4.08 ± 0.30 (3.72 – 4.4)
Length of body whorl	3.84 ± 0.18 (3.56 – 4.00)	4.25 ± 0.34 (3.76 – 4.48)
Length of aperture	3.0 ± 0.14 (2.80 – 3.20)	3.13 ± 0.14 (3.08 – 3.28)
Width of aperture	2.84 ± 0.5 (2.36 – 3.52)	2.98 ± 0.25 (2.64 – 3.24)

TABLE 5. Length (mm) or number of non-neural structures of *Potamolithus ribeirensis*. $\bar{X} \pm$ standard deviation (range). N = number of specimens. * = significant difference between sexes, $P \leq .05$.

	N	♂	N	♀
Body	5	7.24 ± 0.36 (6.80 – 7.80)	5	7.44 ± 0.77 (6.6 – 8.4)
Buccal mass	5	1.08 ± 0.07 (0.96 – 1.14)	5	1.21 ± 0.14 (1.06 – 1.4)
Gonad	5	3.52 ± 0.33 (3.10 – 4.00)	5	1.51 ± 0.10* (1.4 – 1.6)
Mantle cavity	6	2.34 ± 0.15 (2.20 – 2.60)	5	2.44 ± 0.22 (2.20 – 2.80)
Ctenidium	6	2.11 ± 0.15 (1.94 – 2.36)	5	2.14 ± 0.23 (2.00 – 2.56)
Gill filaments: number	6	26.50 ± 2.5 (23 – 29)	5	27.8 ± 5.6 (20 – 32)
Penis	5	1.96 ± 0.23 (1.70 – 2.18)		—
Prostate	5	1.33 ± 0.13 (1.20 – 1.50)		—
Pallial oviduct		—	5	2.38 ± 0.36 (2.1 – 3.0)
Bursa copulatrix		—	6	1.01 ± 0.15 (0.86 – 1.3)
Digestive gland		—	5	3.78 ± 0.44 (3.2 – 4.4)
Seminal receptacle		—	6	0.52 ± 0.09 (0.4 – 0.62)

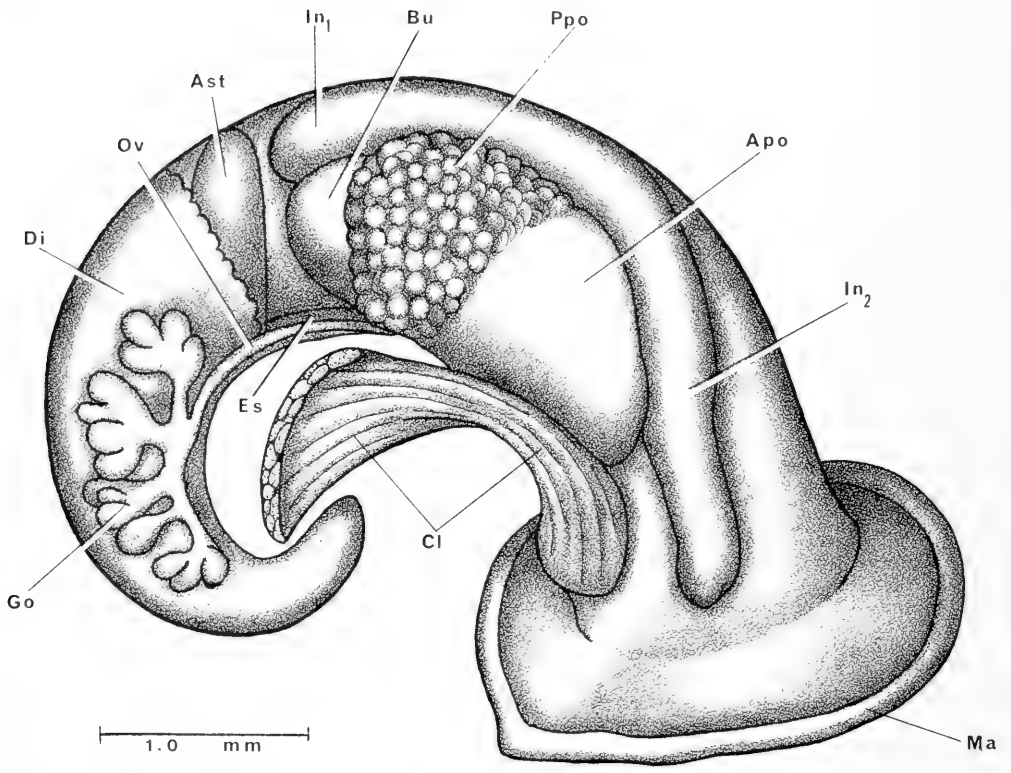

FIG. 7. Uncoiled female without head and with only kidney tissue removed. Note the pallial oviduct (Apo-Ppo) moved over onto the columellar muscle. Note the digestive gland (Di) covering the stomach up to the anterior chamber (Ast). Apo, anterior pallial oviduct = capsule gland; Ast, anterior chamber of stomach; Bu, bursa copulatrix; Cl, columellar muscle; Di, digestive gland; Es, esophagus; Go, gonad; In_1, posterior intestine; In_2, anterior intestine; Ma, mantle edge; Ov, oviduct; Ppo, posterior pallial oviduct = albumen gland.

indicates, the complex has been lifted up and to the left, away from the columellar muscle, to reveal the pericardium (Pe). A portion of the ventral half of the pallial oviduct is removed to expose the interior of the albumen gland (Ppo) and capsule gland (Apo).

The bursa copulatrix (Bu) and posterior half of the albumen gland (Ppo) are ventral to the style sac (Sts, Fig. 9) and loop of intestine (Fig. 7, In_1) encircling the anterior end of the mantle cavity (Emc, Fig. 8A). The bursa copulatrix complex has a common opening with the albumen gland and ventral channel (Vc) at the posterior end of the Vc. This common opening is just posterior to the posterior end of the mantle cavity (Fig. 8A).

The ventral channel is open to the pallial oviduct but it is not a simple ciliated gutter or channel directly opening into the cavity of the pallial oviduct. Rather, it is folded around towards the left dorsal aspect of the pallial oviduct as shown (Fig. 8A–C). The dashed lines across the pallial oviduct (Fig. 8A) indicate where slices were made through the anterior pallial oviduct. The corresponding cross sections are shown in Figs. 8B, C respectively.

There is a thickening along the left ventrolateral edge of the capsule gland lumen where the gland wall folds under and merges into the wall of the ventral channel. This elongate rod of tissue has been described in the literature as a longitudinal fold. In *Hydrobia truncata* studied by one of us (Davis) this fold is best seen by histological section as it is not much thickened and the laterally displaced ventral channel is not prominent. The ventral channel is tightly pressed against the wall of the pallial oviduct. In *Potamolithus ribeirensis* the fold is much thickened and rounded. It is

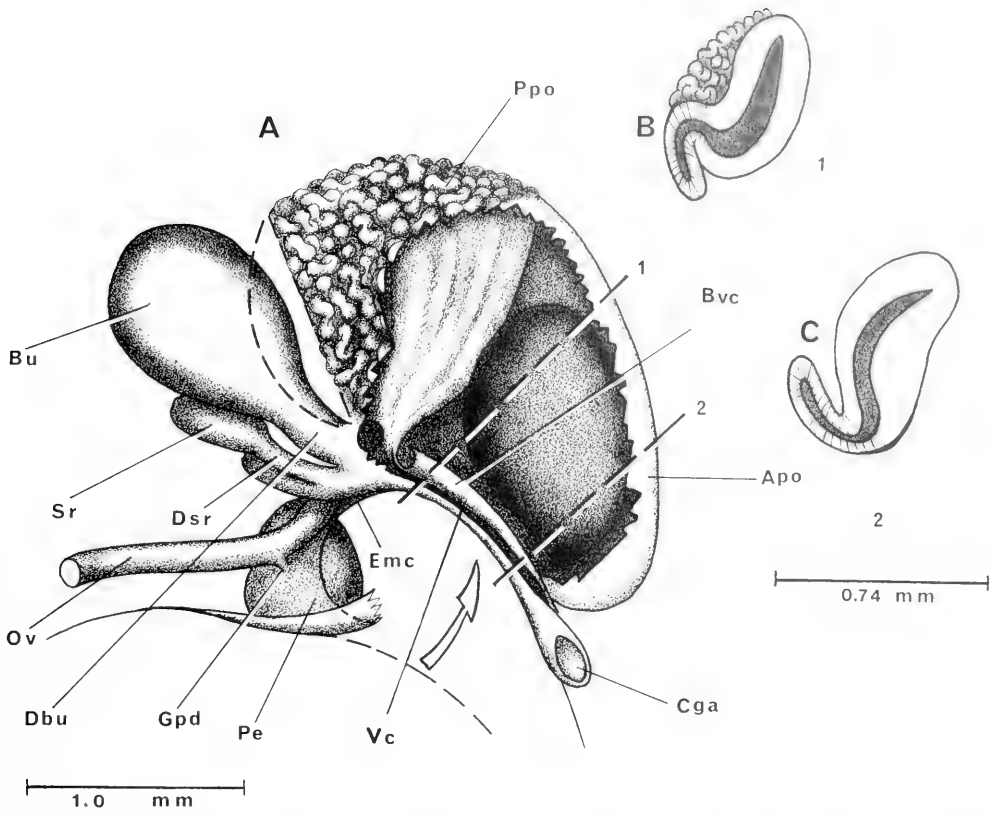

FIG. 8. Female reproductive system in same orientation as Fig. 7. As indicated by the arrow, the pallial oviduct is lifted up and away from the edge of the columellar muscle (dashed line). A section of the albumen gland (Ppo) is cut away to reveal that part of the bursa copulatrix (Bu) covered by the Ppo. A section of the ventral wall of the pallial oviduct is removed to show the interior; in particular, the depressed oval cavity of the capsule gland (Apo). The thickened core of tissue called the bolster of the ventral channel (Vc) results from the fold of the pallial oviduct wall merging with the inner wall of the ventral channel (Vc).

The dashed lines numbered 1 and 2 in Fig. A show where the pallial oviduct was cross sectioned (Figs. B and C). In section B one is looking posteriorly; in section C one is looking anteriorly. The white part of the sections is glandular tissue of the posterior-capsule gland and anterior section of capsule gland respectively. The lined portion of the section indicates the epithelial tissue of the ventral channel (Vc). Note that the Vc in C has separated from the wall of the Apo.

Apo, anterior pallial oviduct = capsule gland; Bvc, bolster of ventral channel; Bu, bursa copulatrix; Cga, common genital aperture; Dbu, duct of bursa copulatrix; Dsr, duct of seminal receptacle; Emc, posterior end of mantle cavity; Gpd, gonopericardial duct; Ov, oviduct; Pe, pericardium; Ppo, posterior pallial oviduct; Sr, seminal receptacle; Vc, ventral channel.

prominent in gross dissection when the pallial oviduct is cut in cross section with iridectomy scissors and examined (under dilute Bouins solution) under 50× magnification. This much thickened longitudinal fold is here called the bolster of the ventral channel (Bvc, Fig. 8A). At the anterior end of the ventral channel, the walls merge to form an enclosed tube. The enclosed tube and common genital aperture are on the floor of the mantle cavity beside the neck (Fig. 6). The anterior end of the capsule gland can be slightly posterior to the genital aperture, abreast of it, or developed to extend beyond it to the interior edge of the wide border (Fig. 6). There is *no* anterior opening of the capsule gland. When the capsule gland was opened a smooth hollow depression could be seen (Fig. 8A) contrasting with the folded

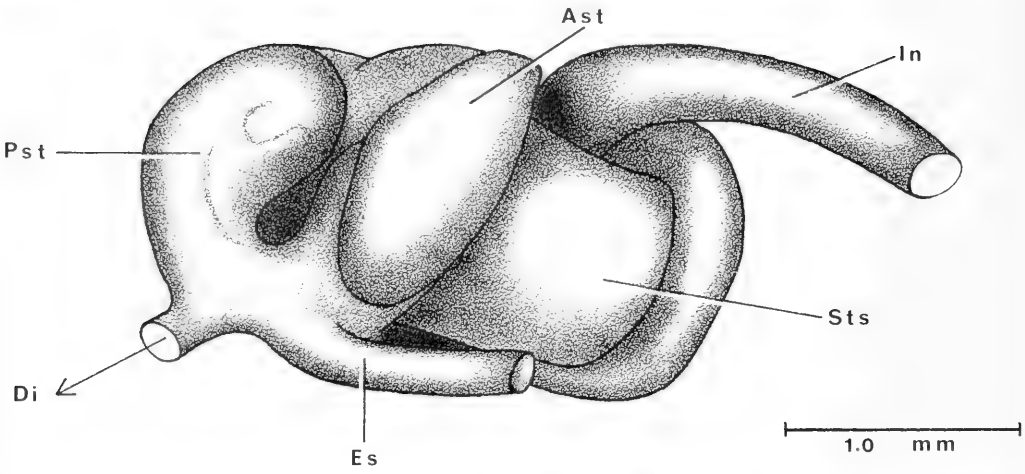

FIG. 9. Ventral surface of the stomach. Ast, anterior chamber; Di, opening to digestive gland; Es, esophagus; In, intestine; Pst, posterior chamber; Sts, style sac.

glandular elements crowding the albumen gland (Ppo).

The bursa complex is understood by comparing Figs. 7, 8A, and 10A, B. The bursa copulatrix (Bu) is ¾ covered ventrally by the albumen gland. The albumen gland is shown cut away in Fig. 8A. The bursa is a relatively large, sac-like organ, 42.4% the length of the pallial oviduct. The seminal receptacle (Sr) is pressed against the bursa, as shown in Fig. 8A, in 90% of the individuals; in 10%, it is dorsal to the bursa and thus out-of-sight, given the orientation of organs in Fig. 8A. The duct of the seminal receptacle (Dsr) is relatively elongate, connecting to the oviduct before the oviduct joins the duct of the bursa (Dbu) at the opening to the common genital groove (Vc).

The seminal receptacle is pulled away from the bursa (Fig. 10A) to show the wide coil of the oviduct. The oviduct coil is not pigmented. A section of the coil directly posterior to the duct opening of the seminal receptacle glistens under direct illumination. The extent of

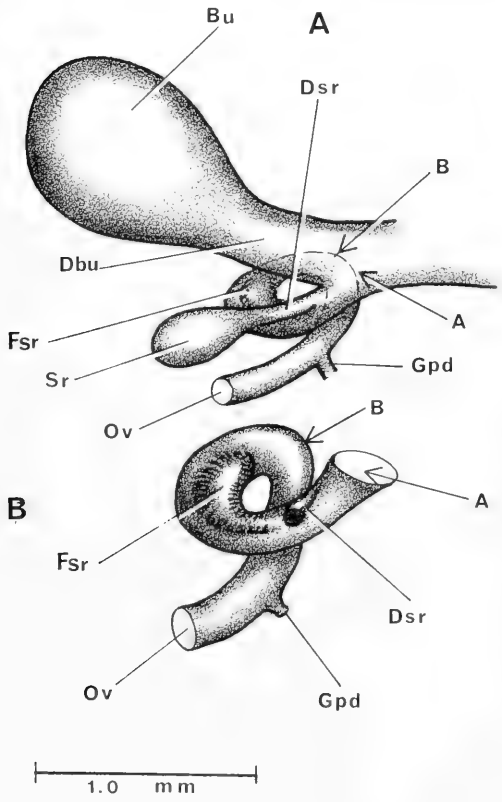

FIG. 10. The bursa copulatrix complex in the same orientation as Figs. 7, 8. A. The seminal receptacle (Sr) has been pulled away from the normal position snug against the left ventrolateral, or dorsolateral edge of the bursa copulatrix (Bu) to show the oviduct coil circling dorsal to the bursa and duct of the bursa (Dbu). B. The duct of the bursa was cut at point A to remove the bursa from obstructing a view of the oviduct coil. The seminal receptacle was removed for the same purpose. The differentially-staining area called a false seminal receptacle is shown (Fsr). A, B, points for orientation, comparing Figs. 10A and 10B; Bu, bursa copulatrix; Dbu, duct bursa copulatrix; Dsr, duct seminal receptacle; Fsr, false seminal receptacle; Gpd, gonopericardial duct; Ov, oviduct; Sr, Seminal receptacle.

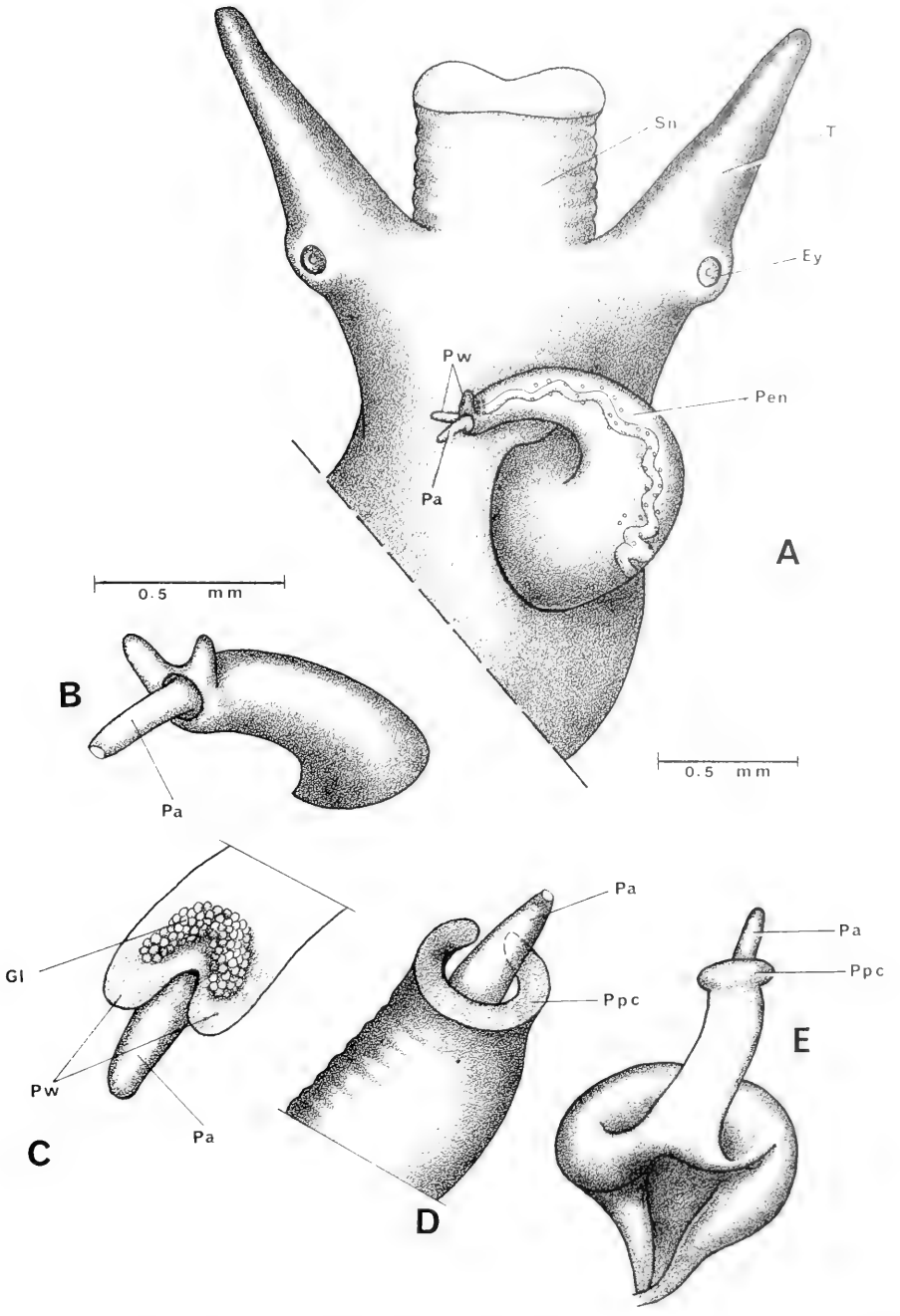

FIG. 11. Male reproductive system: A. Head with typically coiled penis. B. Enlarged view of penis anterior. C. Dorsal view of penis showing typical arc of glands (Gl) and notched appearance over the papilla (Pa) caused by the configuration of the preputial wings (Pw). D. Ventral view of tip of penis showing thickened collar called the preputial collar (Ppc) surrounding the papilla (Pa). E. The penis in Fig. A folded to the left to show the two massive columns of muscle rooting the penis into the columellar muscle. Note the concavity caused by the thickened rolls of muscles and the shaft of the penis. Ey, eye; Gl, glands; Pa, papilla (eversible); Pen, penis; Ppc, preputial collar; Pw, preputial wings; Sn, snout; T, tentacle.

this glistening region is revealed by staining with methylene blue; it takes on a darker blue than the oviduct itself. This distinct region of the oviduct is clearly shown by cutting away the bursa and Sr (Fig. 10B). At first this particular region of the oviduct appeared as a secondary seminal receptacle within the oviduct coil. With histological examination, however, no such Sr_2 was discerned. In this cavernous U-shaped part of the oviduct a section of wall with cuboidal non-ciliated cells contrasted with the remaining wall of columnar ciliated cells. While there were oocytes in the ovary, no sperm were seen in the seminal receptacle, bursa copulatrix, or coil of the oviduct of the eight females sectioned. This was apparently not the reproductive season.

There is a discrete gonopericardial duct (Gpd, Figs. 8A, 10A, B).

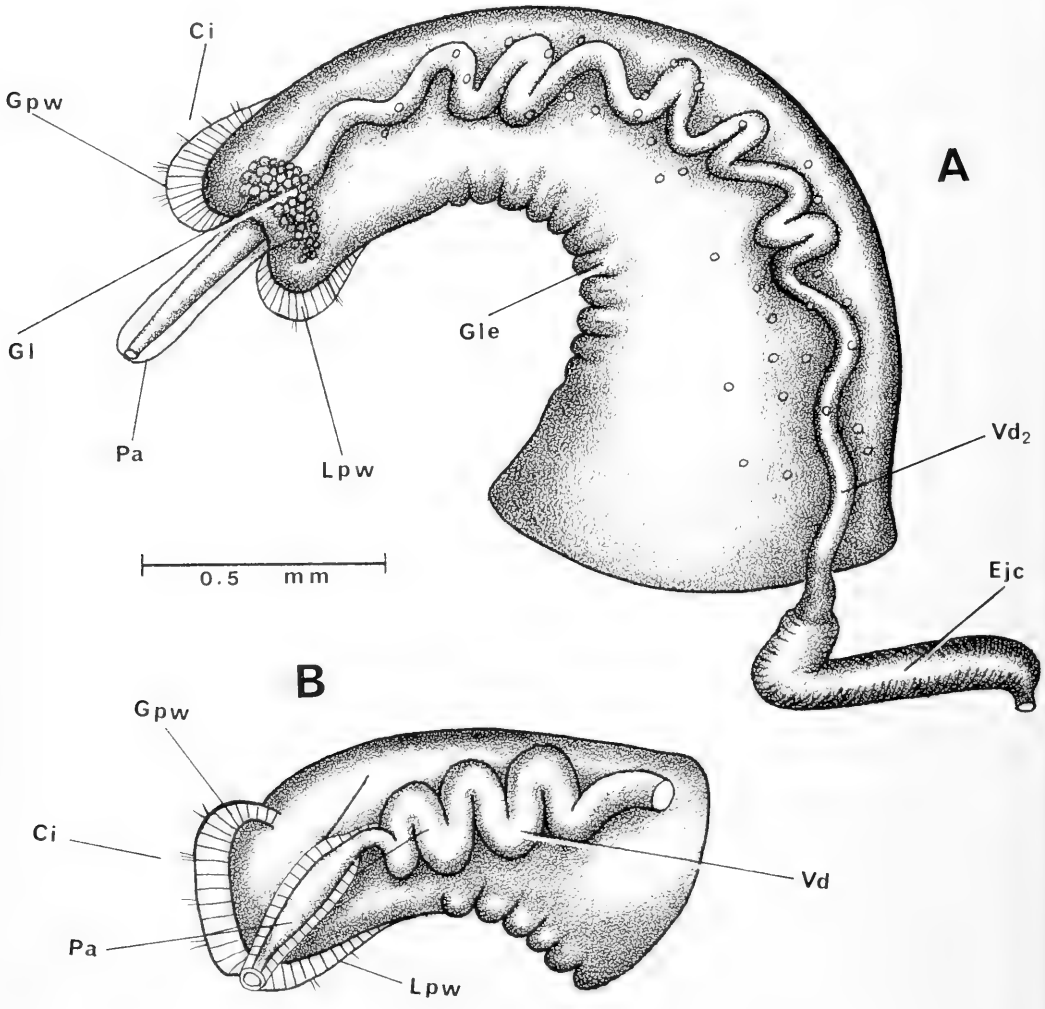

FIG. 12. Penis on a slide under a coverslip viewed by compound microscope. A. Papilla out; B. Papilla withdrawn. White dots along penis (in A) are inclusions appearing white under direct illumination and black with transmitted light. Ci, cilia; Ejc, ejaculatory duct (under the epithelium of the neck); Gpw, greater preputial wing; Gl, glandular patch; Gle, glandular edge of the penis; Lpw, lesser preputial wing; Pa, papilla; Vd, vas deferens; Vd_2, vas deferens from prostate through the penis.

Male reproductive system

Organ dimensions are given in Table 5. The head-neck region of a male is shown (Fig. 11), displaying the prominent penis coiled in typical fashion. In the living snail the unique feature is the wing-like preputial structures here called preputial wings (Pw). These project as shown with the penis in the coiled position. The penial papilla is relatively long and can be retracted within the penis sheath.

The base of the penis differs from that of any other hydrobioid we have examined. Lifting the blade of the penis, as seen in Fig. 11A, and pulling it to the left exposes the base as seen in Fig. 11E. The base is rooted by a horseshoe shaped muscle, i.e. there is a shallow concave hollow within the arc of muscle as shown.

Depending on how the tip of the penis is observed in the living animal, the preputium takes on different aspects. Looking down on the preputial wings, the preputium looks as if there were a U-shaped hollow at the end of the penis sheath (Fig. 11C). Turning the penis over 180°, the preputium looks like a thickened collar through which the papilla is extended (Fig. 11D).

When the penis is examined with a compound microscope (on a slide under a coverslip), the result is seen in Fig. 12A. The preputial wings are flattened and appear as areas of extended columnar cells. There are irregular tufts of stiff vibratile cilia arising from the preputial wings. The tip of the penis is shown with papilla extended (Fig. 12A) and withdrawn (Fig. 12B). Near the tip of the penis, behind the preputial ring, is a concentration of glands (Gl) beneath the surface of the epithelium. These are spherules that look black under transmitted light. The vas deferens undulates through the penis near the convex edge (Fig. 12A). The thickened ejaculatory duct (Ejc) is not embedded in the base of the penis, but in the neck.

The uncoiled male without head and kidney tissue is shown in Fig. 13A. The prostate is squeezed against the columellar muscle. Distinctive features are: 1) the gonadal lobes are massive; the gonad does not have a slender, delicate branching system; 2) the anterior gonadal lobes are ventral to the posterior chamber of the stomach, i.e. the gonad is not restricted behind the stomach; 3) the gonad-vas deferens configuration is otherwise of the general type (Davis, 1980); 4) the seminal vesicle (Sv) was hardly developed in 90% of the males dissected. There was hardly a single fold or loop of the vas deferens (Vd_1) near the gonad signifying the seminal receptacle (Fig. 13A). In 10% (older individuals as evidenced by shell parietal callus development) the seminal vesicle was a pronounced coil of tubes dorsal and obliquely angled to the gonad (Fig. 13B); 5) the prostate overlies the posterior end of the mantle cavity; 6) the posterior prostate is ventral to the entire style sac, thus hiding it from view in Fig. 13A; 7) the posterior prostate is flattened and fan-shaped, contrasting with the inflated bean-shape of the rest of the prostate; 8) the anterior vas deferens (Vd_2) leaves the prostate as shown (Fig. 13A, B); the generalized condition (Davis, 1980).

Digestive system

Of importance here are the salivary glands, stomach, and radula. The salivary glands are paired, each gland a simple elongate tube that lies dorsal to the nerve ring. The stomach is shown in ventral view (Fig. 9) with a characteristically raised section of the anterior chamber (Ast), and a distinctive shape of the posterior chamber (Pst). The posterior chamber has a smooth, rounded posterior contour, i.e. there are no folds or distended projections of this chamber (contrast the so-called appendix of the stomach—e.g. Radoman, 1977, fig. 2A, *Hydrobia acuta*). One can see, inside the ventral surface of the posterior chamber, raised ridges shown as dark lines in Pst (Fig. 9).

The radula is typically taenioglossate (Fig. 14). Radular statistics are given in Table 6 and cusp formulae in Table 7. Note the concave hollow ventral to the basal cusps of the central tooth (Fig. 14B); the lateral angle of the central tooth is widely spread; the innermost pair of basal cusps arise from the face of the tooth. There is a pronounced posterior projection of the face of the lateral tooth (Fig. 14C, D).

Nervous system

The nervous system is standard rissoacean. Statistics on neural structures are given in Table 8. Of note are: 1) the elongate cerebral commissure; 2) the elongate pleuro-supraesophageal connective yielding an RPG ratio of 0.58; 3) the relatively long pleuro-subesophageal connective. This is an open nervous sytem, i.e. the ganglia are separated by elongate connectives and commissures.

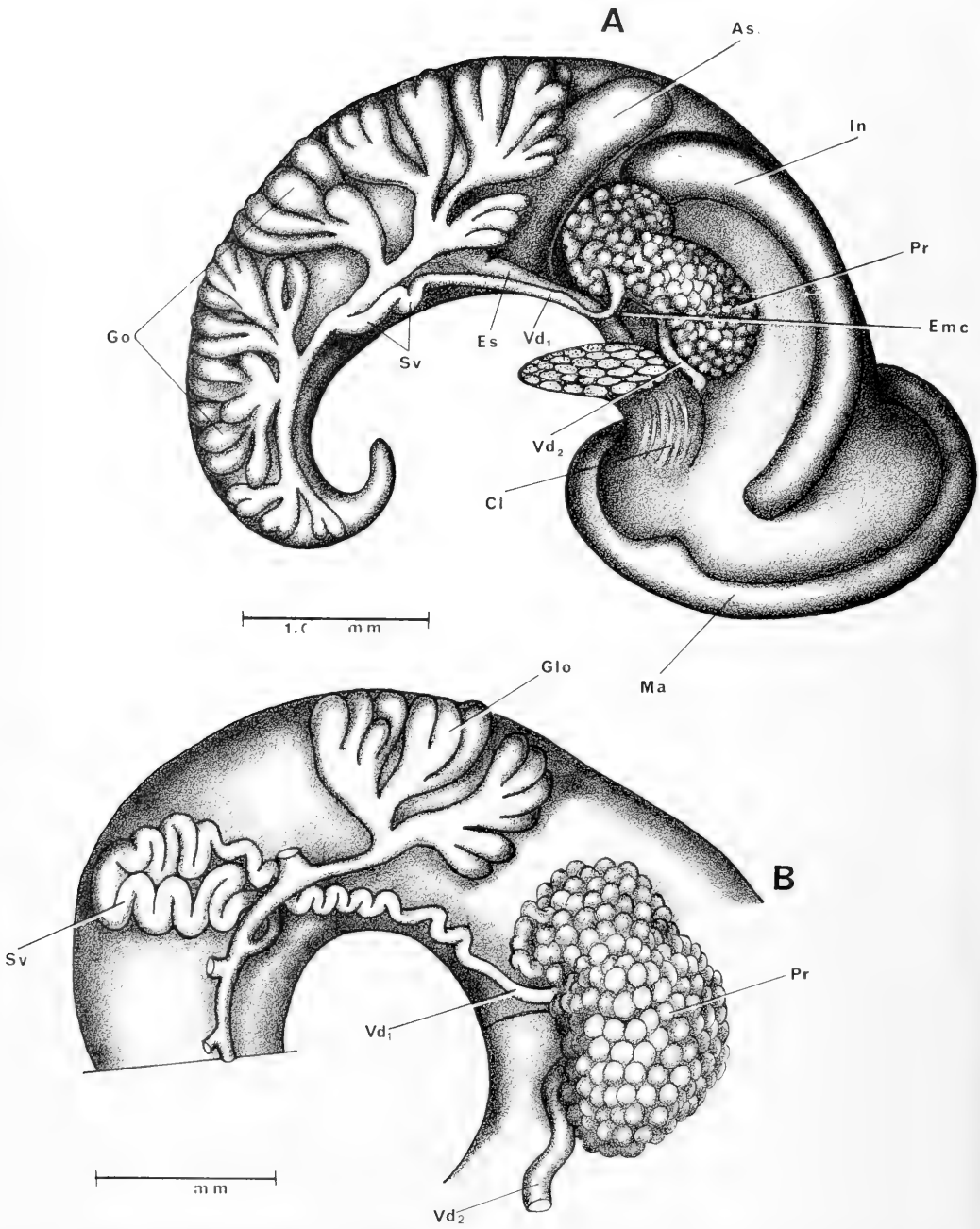

FIG. 13. Male reproductive system. A. Ventral view of uncoiled snail without head and with kidney tissue removed. B. Some lobes of the gonad are removed to show the position and nature of coils of the seminal vesicle (Sv). Ast, anterior chamber of the stomach; Emc, posterior end of the mantle cavity; Es, esophagus; Cl, columellar muscle; Glo, gonadal sperm-producing lobe; Go, gonad; In, intestine; Ma, mantle edge; Pr, prostate; Sv, seminal vesicle; Vd_1, posterior vas deferens; Vd_2, anterior vas deferens.

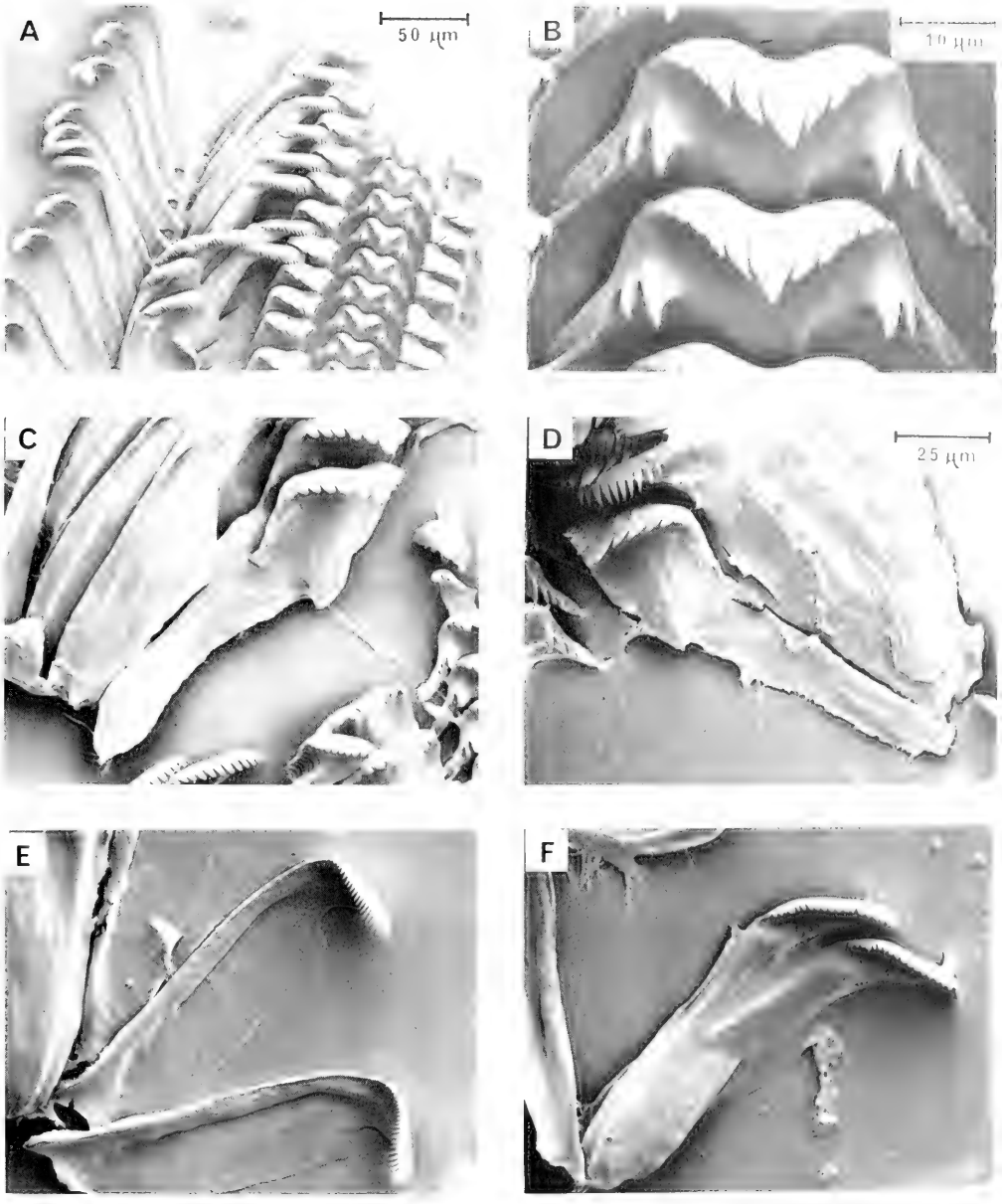

FIG. 14. Radula. A, portion of radular ribbon; B, central tooth; C, left lateral tooth; D, right lateral tooth; E, outer marginal. F, inner marginal. C–F are at the same magnification.

TABLE 6. Radular characteristics of *Potamolithus ribeirensis*. Measurements in mm. $\bar{X}$, mean; ± standard deviation; ( ), range; N = number studied.

Radular character	♂	♀	♂ + ♀
Length	1.30 ± 0.10 (1.13 – 1.37) N = 5	1.48 ± 0.11 (1.27 – 1.59) N = 8	1.41 ± 0.14 (1.13 – 1.59)
Width	0.20 ± 0.01 (0.18 – 0.22) N = 5	0.22 ± 0.02 (0.20 – 0.25) N = 8	0.20 ± 0.02 (0.18 – 0.25)
Rows (no.)	93.2 ± 8.2 (80 – 102) N = 5	96.5 ± 6.1 (87 – 106) N = 8	95.2 ± 6.5 (80 – 106)
Rows forming (no.)	6.4 ± 1.5 (5 – 9) N = 5	8.5 ± 2.0 (7 – 13) N = 8	7.7 ± 2.1 (5 – 13)
Central tooth (width)	0.0398 ± 0.003 (0.0370 – 0.0440) N = 5	0.0460 ± 0.002 (0.0440 – 0.0480) N = 5	0.0429 ± 0.004 (0.0370 – 0.0480)

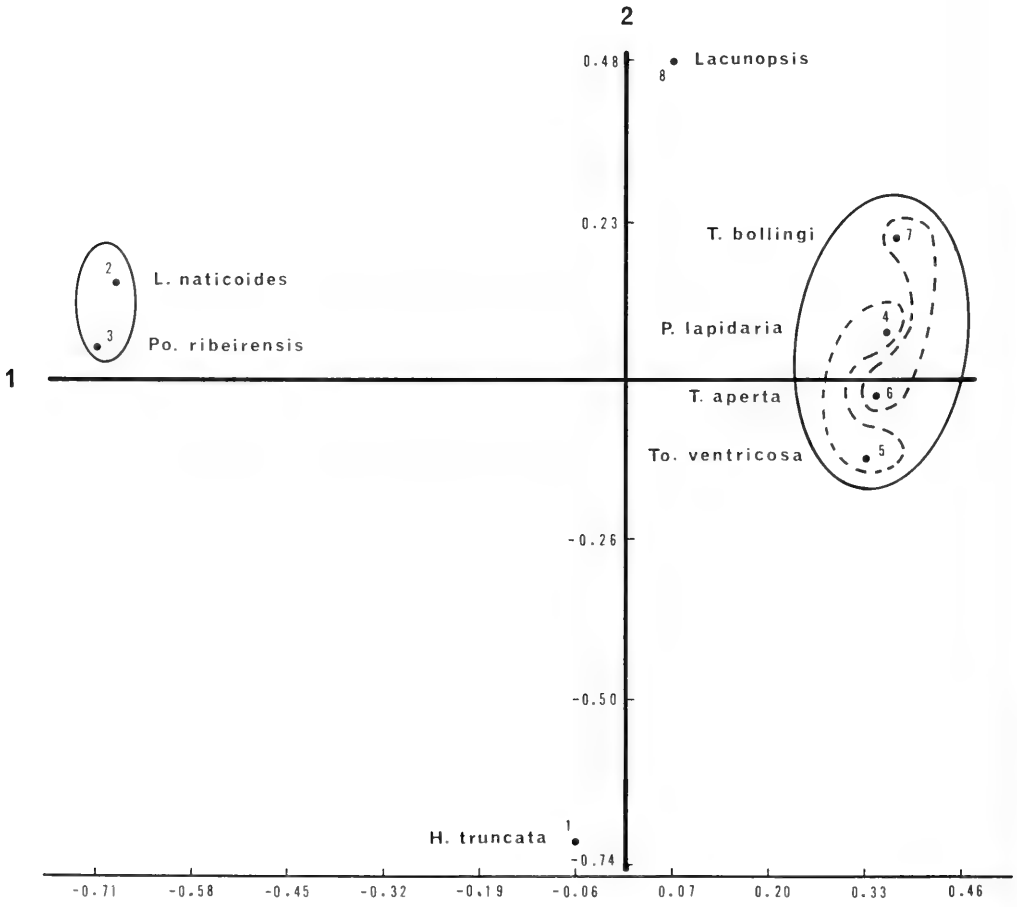

FIG. 15. Ordination diagram following three-dimensional scaling, showing the distribution of eight taxa along axes 1 and 2. Taxa are grouped into sets (solid lines) and sub-sets (dashed lines). See text for explanation.

TABLE 7. Formulae for the most common cusp arrangements of the four radular teeth types of *Potamolithus ribeirensis*. Ten radulae were examined.

Tooth	Formula	% teeth with formula	Other types
Central	$\frac{5\text{-}1\text{-}5}{2(3)\text{-}(3)2^*}$	100%	—
Lateral	4-1-4 one side 5-1-5 other side	30%	5-1-5 one side 4-1-5 other side
	5-1-5	40%	4-1-5
Inner Marginals	25	21%	22, 28-32
	26	18%	
	23, 24, 27	42%	
Outer Marginals	30	20%	24-27, 31
	29	18%	34-36
	32	12%	
	33	12%	
	28	11%	

*The third basal cusp is vague and insignificant in a few specimens, a raised ridge in some specimens, and was not discerned in most specimens.

TABLE 8. Length (mm) or ratio of neural structures of *Potamolithus ribeirensis*. N = number. $\bar{X}$, mean. ± standard deviation (range).

	N	
Cerebral ganglion	5	0.34 ± 0.09 (0.28 – 0.50)
Cerebral commissure	5	0.17 ± 0.06 (0.08 – 0.24)
Pleural ganglion: right (1)	5	0.14 ± 0.02 (0.12 – 0.16)
Pleural ganglion: left	5	0.15 ± 0.01 (0.14 – 0.16)
Pleuro-supraesophageal connective (2)	5	0.46 ± 0.09 (0.36 – 0.6)
Supraesophageal ganglion (3)	5	0.18 ± 0.02 (0.16 – 0.20)
Osphradiomantle nerve	5	0.07 ± 0.07 (0 – 0.2)
Pleuro-subesophageal connective	5	0.04 ± 0.03 (0 – 0.08)
Subesophageal ganglion	5	0.18 ± 0.02 (0.16 – 0.20)
Pedal ganglion	5	0.25 ± 0.04 (0.22 – 0.28)
Pedal commissure	4	0.04 ± 0.03 (0 – 0.08)
Statocyst diameter	1	0.12
Osphradium	4	0.87 ± 0.09 (0.80 – 1.0)
RPG ratio*	5	0.58 ± 0.04 (0.53 – 0.63)

*2/1 + 2 +3

RESULTS: ANALYSIS OF RELATIONSHIPS

Multivariate analysis

The data matrix is shown in Table 9. Three ordination diagrams compare OTU placements when principal components (PC) 1 × 2, 1 × 3, and 2 × 3 are compared (Figs. 15–17). PC one (axis 1) has 55.08% of the variance; PC two has 32.70%; PC three has 12.22%. The stress after fifty iterations was 0.008. The matrix correlation was 0.941.

In the principal components analysis the first five PCs involved 94.19% of the variance as follows: 1) 35.51%; 2) 23.95%; 3) 15.65%; 4) 11.90%; 5) 7.18%. Character loading on the first four PCs is given in Table 10.

PC one: Some fifteen characters load on factor one with scores of 0.482 or more; ten characters load with scores >0.70. This axis is structured with snails having globose shells (character 1), wide parietal callus (2), keel formation (3), a powerful foot (6), gonad overlapping the stomach (17), pallial oviduct with a ventral channel (18), with opening of pallial oviduct lateral, not at the anterior end (19), eggs without sand covering (30)—to the far left. Snails to the far right (OTUs 4–7) have ovate-conic to turreted shells, without keels, without squat heads or powerful feet, with gonads posterior to the stomach, with spermathecal duct separate from the pallial oviduct, with oviduct opening at the anterior end, and with eggs covered by sand.

Lacunopsis (OTU no. 8) shares shell and

TABLE 9. Matrix of 8 taxa (OTUs) by 32 characters for a multivariate analysis to assess relationships among taxa and characters. In (0,1), 0 = does not have, 1 = has; * presumed character state given data or lack of data in the literature.

	Taxa							
Character	1 *Hydrobia truncata*	2 *Lithoglyphus naticoides*	3 *Potamolithus ribeirensis*	4 *Pomatiopsis lapidaria*	5 *Tomichia ventricosa*	6 *Tricula aperta*	7 *Tricula bollingi*	8 *Lacunopsis* all spp.
1. Shell: turreted (0), ovate-conic (1), globose (2)	1	2	2	0	0	1	1	2
2. Shell: wide parietal callus/ shelf (0,1)	0	1	1	0	0	0	0	1
3. Shell: tends to form a keel (0,1)	0	1	1	0	0	0	0	1
4. Head: broad, squat (0,1)	0	1	1	0	0	0	0	1
5. Tentacles: elongate-slender (0,1)	1	1	0	1	1	1	1	0
6. Foot: wide, powerful (0,1)	0	1	1	0	0	0	0	1
7. Foot: pedal crease (0,1)	0	0	0	1	1	0	0	0
8. Mantle: pallial tentacle (0,1)	1	0	0	0	0	0	0	0
9. Tentacle: left has hypertrophied cilia (0,1)	1	0	0	0	0	0	0	0
10. Osphradium: elongate (0,1)	1	1	1	0	0	1	0	1
11. Central tooth: 3 or more pairs of basal cusps (0,1)	0	1	1	1	1	1	1	1
12. Central tooth: 1 or more pair basal cusps from face of tooth (0,1)	0	1	1	1	1	1	1	1
13. Penis with eversible papilla (0,1)	0	0	1	0	0	1	0	0
14. Penis wide, blunt (0,1)	0	1	0	0	0	0	0	0
15. Prostate, pallial oviduct squeezed over on columellar muscle	0	1*	1	0	0	0	0	1
16. Ejaculatory duct: none (0), in neck (1), in base of penis (2)	0	0*	1	2	0	2	2	0

17. Part of ♂ gonad overlaps posterior chamber of stomach (0,1)	1	1*	1	0	0	0	0	0
18. Female: sperm enters system via duct separated from pallial oviduct (0,1)	0	0	0	1	1	1	1	1
19. Opening of pallial oviduct at anterior tip (0,1)	0	0	0	1	1	1	1	1
20. Ventral channel: none (0), small relative to size of pallial oviduct; tightly bound to medial ventro-lateral edge of pallial oviduct (1), large relative to size of pallial oviduct and not tightly bound to pallial oviduct (2)	1	1*	2	0	0	0	0	0
21. Oviduct with bolster (0,1)	0	0	1	0	0	0	0	0
22. Oviduct and duct of bursa join directly (0) or via sperm duct (1)	0	0	0	1	1	0	0	0
23. Seminal receptacle present (0) or replaced by secondary seminal receptacle (1)	0	0	0	0	0	0	0	1
24. Oviduct coil (0,1)	1	1	1	1	1	0	0	0
25. *Lacunopsis* type oviduct twist (0,1)	0	0	0	0	0	0	1	1
26. Spermathecal duct enters pericardium (0,1)	0	0	0	0	0	0	1	1
27. Bursa elongated (0,1)	0	1	1	0	1	1	0	0
28. Sperm enters ♀ at anterior end of mantle cavity (0,1)	1	1	1	1	1	0	0	0
29. Nuchal node present (0,1)	0	0	1	0	0	0	0	0
30. Eggs covered by sand grains (0,1)	1	0	0	1	1	1	1	1
31. Posterior chamber of stomach with fold and posterior prominence (= "appendix") (0,1)	1	0	0	0	0	0	0	0
32. Nervous system concentrated (0,1)	0	1	0	0	0	0	0	0

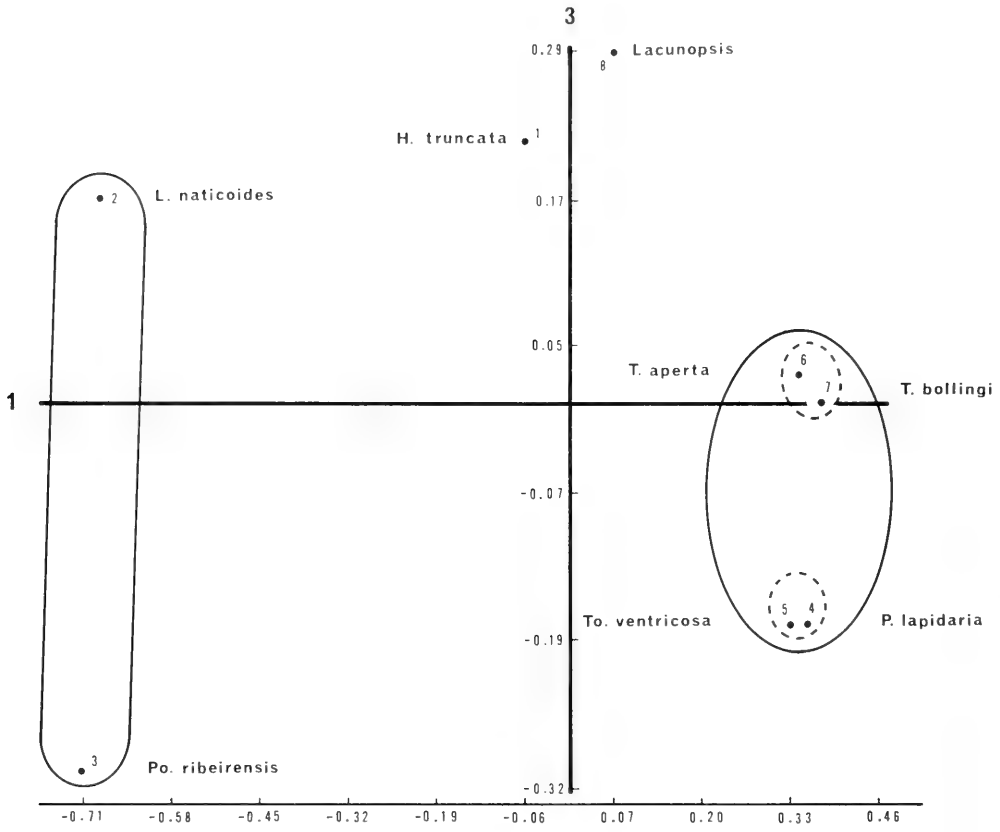

FIG. 16. As in Fig. 15, except axes are 1 × 3.

TABLE 10. Factor loading of 32 characters for each of the first four principal components that collectively account for 87.01% of the variation in this study.

Character	Principal components			
	1	2	3	4
1	0.827	−0.397	0.354	−0.106
2	0.840	−0.488	0.051	0.169
3	0.576	−0.417	−0.194	0.363
4	0.840	−0.488	0.051	0.169
5	−0.520	0.601	−0.035	0.353
6	0.840	−0.488	0.051	0.169
7	−0.551	0.170	−0.582	0.380
8	0.111	0.806	0.475	−0.269
9	0.111	0.806	0.475	−0.269
10	0.740	−0.008	0.330	−0.284
11	−0.111	−0.806	−0.475	0.269
12	−0.111	−0.806	−0.475	0.269
13	0.265	−0.211	−0.431	−0.700
14	0.549	0.073	0.029	0.709
15	0.840	−0.488	0.051	0.169
16	−0.482	−0.227	−0.289	−0.310
17	0.864	0.481	0.004	−0.056
18	−0.864	−0.481	−0.004	0.056

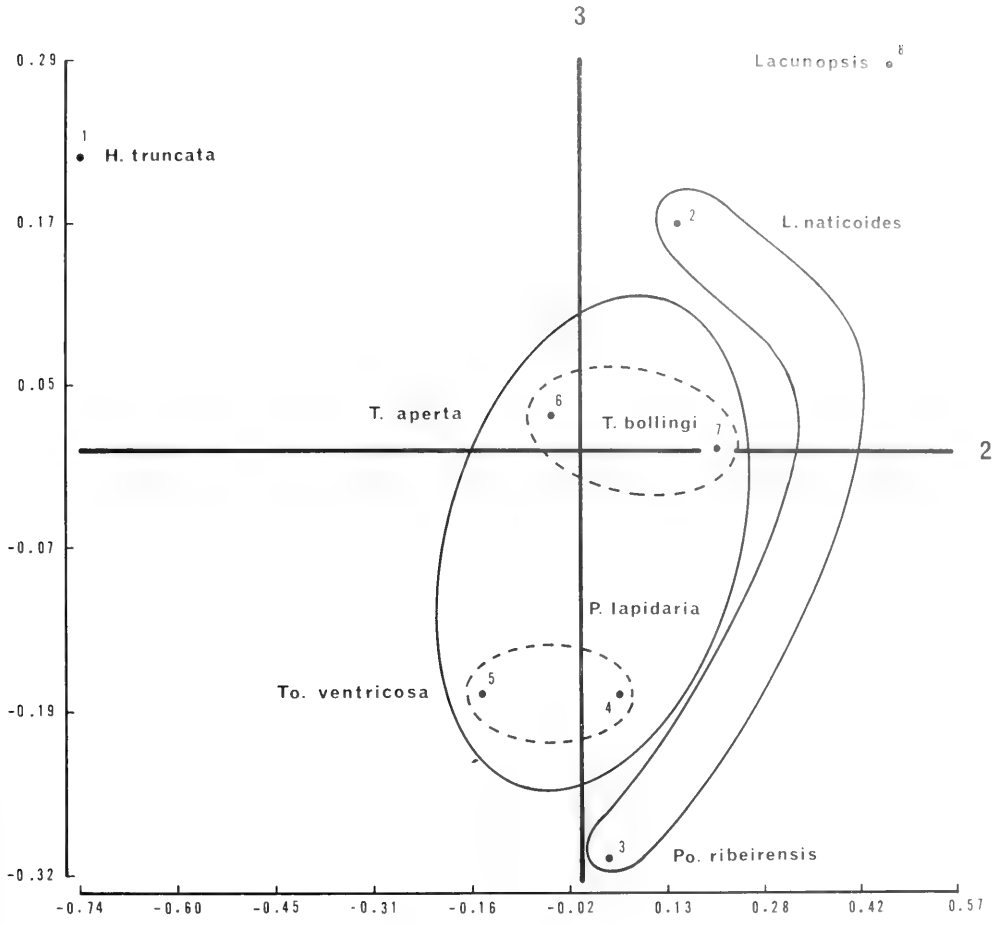

FIG. 17. As in Fig. 15, except axes are 2 × 3.

TABLE 10 (Continued)

Character	Principal components			
	1	2	3	4
19	−0.864	−0.481	−0.004	0.056
20	0.875	0.248	−0.230	−0.282
21	0.605	−0.174	−0.498	−0.522
22	−0.551	0.170	−0.582	0.380
23	0.076	−0.614	0.544	0.060
24	0.371	0.634	−0.516	0.285
25	−0.218	−0.630	0.628	−0.017
26	−0.218	−0.630	0.628	−0.017
27	−0.011	−0.094	−0.654	−0.492
28	0.371	0.634	−0.516	0.285
29	0.605	−0.174	−0.498	−0.522
30	−0.881	0.077	0.358	−0.143
31	0.504	0.671	0.385	0.337
32	0.549	0.073	0.029	0.709

head-foot character states with OTUs 2 and 3 at the far left yet shares five character states (17–20, 30) loading heavily on this axis with OTUs 4–7 at the far right. Accordingly, this taxon is to the left of OTUs 4–7. *Hydrobia* (OTU no. 1) shares shell and head-foot character-states with OTUs 4–7, and covers eggs with sand as do OTUs 4–8, but shares character states 17–19, and 22 with OTU nos. 2, 3. Accordingly, this taxon, as with *Lacunopsis*, is to the left of OTUs 4–8.

Character states that relegate *Potamolithus* and *Lithoglyphus* to the far left are those shared or unique to one of them, i.e. the condensed nervous system (32), nuchal node (29), bolster of the pallial oviduct (21), ejaculatory duct in the neck (16), and blunt penis (14). A similar arrangement of ducts involving the pallial oviduct, sperm groove, lateral opening of the pallial oviduct, and duct of the bursa joining the oviduct (22) place OTUs 1–3 to the left of the second axis.

PC two: Eleven characters load on this axis with a score >0.60; four with a score >0.80. At the bottom (*Hydrobia*, OTU no. 1) the character-states are: the mantle has a tentacle (8); the left tentacle has hypertrophied cilia (9); the central tooth has fewer than three basal cusps (usually only one); the stomach has a posterior prominence; the basal cusp arises from the lateral angle, not the face of the tooth (12). Towards the top of axis two snails have no mantle tentacle, are without hypertrophied cilia, the central tooth has three or more basal cusps with the innermost pair arising from the face of the tooth; the stomach does not have a posterior prominence. *Lacunopsis* (OTU no. 8) is at the top of the second factor axis as it alone has accessory seminal receptacles. OTUs 7 and 8 are at the top of the second factor axis, as they alone have a distinctive oviduct twist (contrasted with oviduct coil typical of the Hydrobiinae, Pomatiopsinae, and Lithoglyphinae); they have a spermathecal duct that enters the pericardium, a condition not occurring in the other taxa.

PC three: Eight characters load on this axis with a score >0.50; no score is >0.654. Only four characters score higher on this axis than axes one or two (nos. 7, 13, 22, 27). Interpretation of this axis is weak. Those two taxa (OTUs 3 and 8) with the unique character states are separated at either end of the axis. *Lacunopsis* (OTU no. 8) has the secondary seminal receptacles (23) while *Potamolithus* (OTU no. 3) has the nuchal node (29) and bolster of the pallial oviduct (21). Taxa with the pedal crease (7) are low on the axis (OTU nos. 4 and 5). Otherwise, given the character loading score for this axis, we see no other clear biological interpretation.

Subsets: *Hydrobia* and *Lacunopsis* are not included in subsets. *Potamolithus* and *Lithoglyphus* are in a set clearly separated along axis one from the sets and subsets that group *Pomatiopsis* and *Tomichia* (one subset) and the two species of *Tricula* (other subset).

Character correlations: A number of characters are highly correlated and cluster together at >0.90. The most prominent group involves shell shape (1), degree of parietal callus (2), squat head (4), wide and powerful foot (6), and prostate or pallial oviduct squeezed over towards the columellar muscle (15). The concentrated nervous system (32) and blunt penis (14) are thus correlated. The pallial tentacle (8) is highly correlated with hypertrophied cilia on the left tentacle (9). The pedal crease (7) correlates thus with the sperm duct (22). The central tooth characters are highly correlated (11, 12), as are the *Lacunopsis*-type oviduct twist (25) with the spermathecal duct entering the pericardium (26).

Cladistic analysis

A set-theory nesting of the eight taxa in question on the basis of unique and presumably unreversed characters *sensu* Wilson (1965) is given in Fig. 18. The data for *Lithoglyphus* came from Krull (1935), Krause (1949), and Radoman (1966). The data for the Pomatiopsinae and Triculinae came from Davis, 1979, 1980; Davis & Greer, 1980. The data for *Hydrobia* were summarized in Hershler & Davis (1980), with additional data from Radoman (1973, 1976). The largest set A is defined by the most generalized character-states, i.e. shared by all taxa in the smaller sets. Character-states that serve to define sets are given in Table 11, where the letters correspond to the lettered sets of Fig. 19.

A cladogram was structured from the sets by rooting the tree at A (Fig. 19), a taxon presumably having the generalized characters listed (Tables 11, 12). Derived character-states are gains or losses of certain states numbered 2 to 8 on Fig. 20 and in Table 12. The question marks indicate that we do not have sufficient data to link lineages B_1 and B_2. It has been argued that these lineages do not share an immediate common ancestor A

TABLE 11. Character-states defining sets shown in Fig. 18.

A. Rissoacean grade of organization, including species without pedal tentacles, with closed pallial oviducts, with pallial oviduct comprised of albumen and capsule glands merging directly into each other, with basal cusps on central tooth, with seminal receptacle posterior to bursa copulatrix, where the penis has one duct, where the operculum lacks internal pegs, projections, and calcium smears.

B_1. 1) Sperm carried to bursa copulatrix in a grooved channel or duct open to the pallial oviduct; 2) female genital aperture of posterolateral to anterior end of pallial oviduct.

C_1. 1) One or two pairs basal cusps on rachidian tooth; 2) basal cusps arise from lateral angle; 3) hypertrophied cilia on left tentacle; 4) posterior chamber of stomach with posterior protuberance and fold; 5) shell ovate-conic ..... *Hydrobia s.s.*

C_2. 1) Three or more pairs of basal cusps on rachidian tooth; 2) some of basal cusps arise from face of tooth, not lateral angle; 3) no hypertrophied cilia on left tentacle; 4) stomach without protuberance or fold; 5) shell globose ..... Lithoglyphinae

D_1. 1) Nuchal node on neck of female; 2) pleuro-supraesophageal connective elongate; 3) penis with protractile papilla; 4) penis slender; 5) sperm groove offset, folded around ventral pallial oviduct ..... *Potamolithus*

D_2. 1) No nuchal node; 2) pleuro-supraesophageal connective short or absent; 3) penis without papilla; 4) penis wide and blunt; 5) sperm groove opens directly into lumen of pallial oviduct ..... *Lithoglyphus*

B_2. 1) Sperm carried in enclosed spermathecal duct not connected to the pallial oviduct; 2) opening of female system at anterior end of pallial oviduct.

C_3. 1) Spermathecal duct opens at anterior end of mantle cavity; 2) oviduct coil retained; 3) pedal crease; 4) maintains gonopericardial duct ..... Pomatiopsinae

D_3. 1) Short bursa copulatrix; 2) sperm duct arises from duct of bursa ..... *Pomatiopsis*

D_4. 1) Long bursa copulatrix; 2) sperm duct arises from bursa some distance from anterior end of bursa ..... *Tomichia*

C_4. 1) Sperm enters spermathecal duct from posterior of mantle cavity; 2) no oviduct coil; no pedal crease; 4) no gonopericardial duct ..... Triculinae

D_5. Spermathecal duct does not enter pericardium ..... *T. aperta*

D_6. Spermathecal duct enters pericardium.

E_1. 1) Seminal receptacle standard, joins oviduct; 2) no accessory seminal receptacles ..... Triculinae: *"Tricula" bollingi*

E_2. 1) Standard seminal receptacle lost; 2) with several secondary seminal receptacles ..... *Lacunopsis*

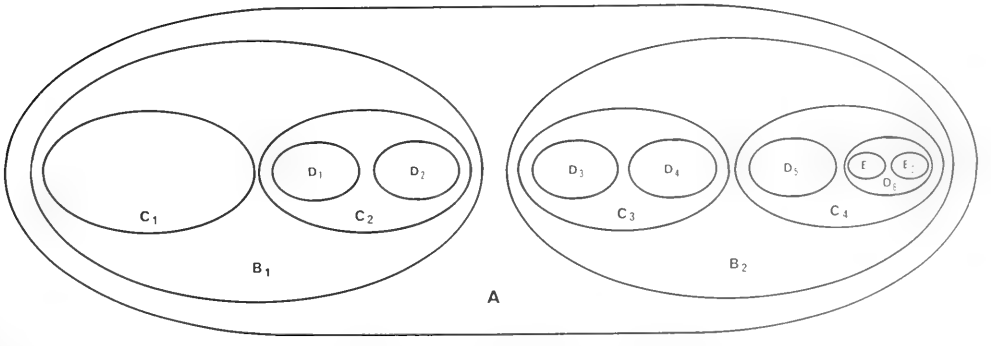

FIG. 18. Set theory solution *sensu* Wilson (1965) to nest taxa on the basis of unique characters given in Table 11.

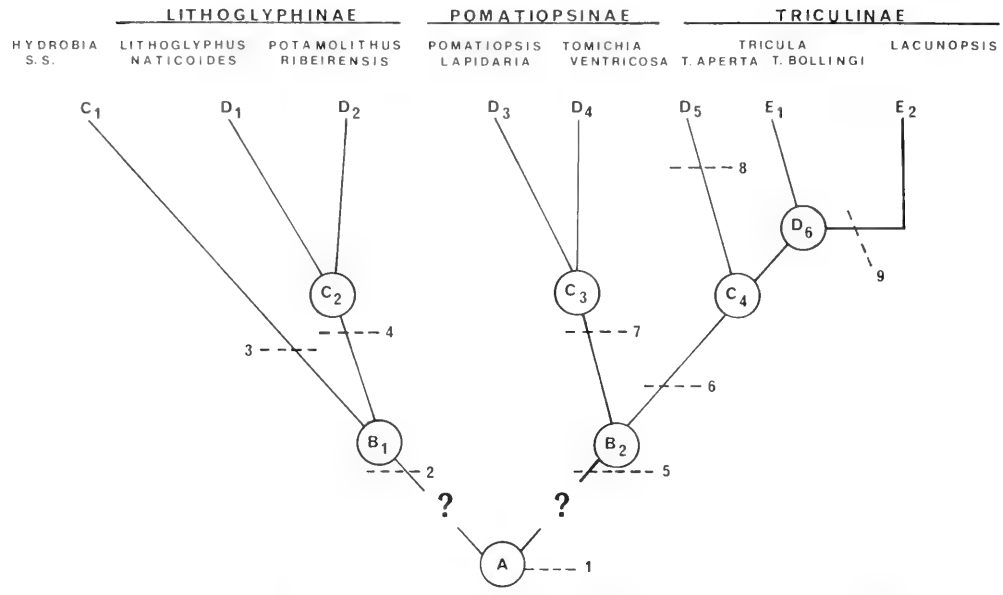

FIG. 19. Cladogram based on rooting taxa of the sets shown in Fig. 18, based on shared derived character-states of Tables 11, 12. The question marks indicate uncertainty about the two lineages sharing an immediate common ancestor (see text).

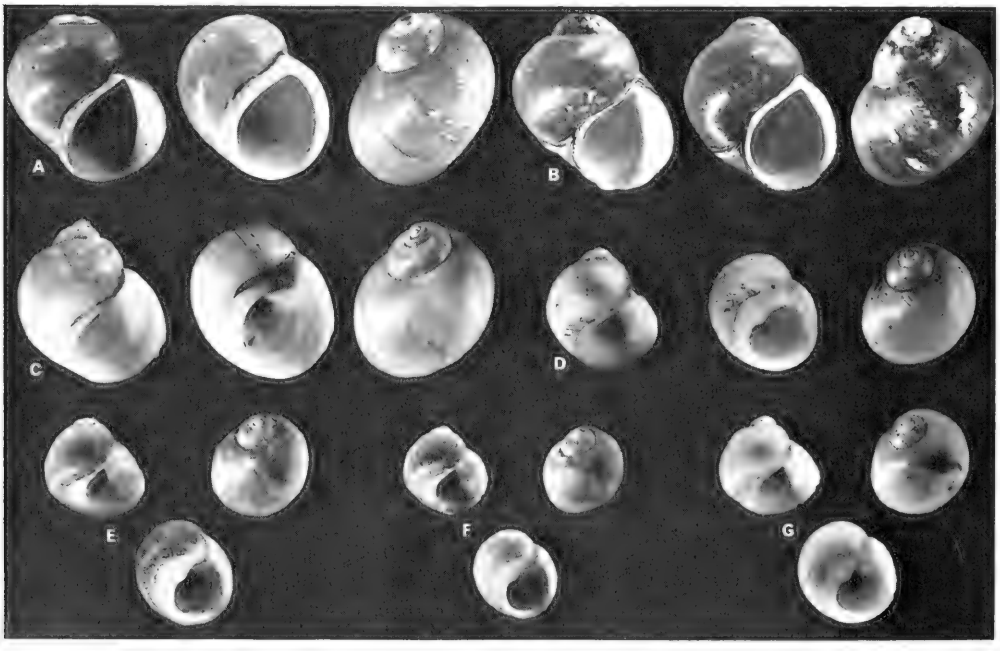

FIG. 20. Shells of species converging on *Potamolithus* in shell shape. A, *Somatogyrus aureus* (ANSP 69284); shell length 6.31 mm; other shells printed at the same magnification; B, *Fluminicola seminalis* (ANSP 175283); C, *Lithoglyphus naticoides* (ANSP 345100); D–G, *Lacunopsis sphaerica* (ANSP 345100).

TABLE 12. Definition of numbered sets of character-states shown in Fig. 19. Numbers 1–2 indicate the maintenance of generalized characters, while 3–9 are sets of shared derived characters. Use with Table 11.

1. Character-states that are generalized and presumed primitive are: shell small (< 5.0 mm), ovate-conic; animal with oviduct coil, with gonopericardial duct, stomach without posterior folds or protuberances of the posterior chamber, central tooth with one pair of basal cusps off lateral angle, seminal receptacle joins the oviduct, pallial oviduct with ciliated gutter to transport sperm, hypertrophied tentacle cilia; with pallial tentacle.
2. Same as 1 above.
3. Development of fold and protuberance (= appendix of Radoman) of posterior stomach chamber.
4. Loss of hypertrophied tentacle cilia, loss of pallial tentacle, increased complexity of basal cusps on central tooth, specialized shell shape.
5. Spermathecal duct separated from pallial oviduct, thus replacing ciliated gutter of pallial oviduct; increased complexity of basal cusps on central tooth of radula (parallel with same trend at 4 above).
6. Loss of gonopericardial duct, loss of oviduct coil, sperm enter female reproductive system at posterior end of mantle cavity (possibly a primitive character found in ancestral taxa at point 5).
7. Step-like mode of progression, pedal crease, suprapedal fold, eyes in enlarged bulges at base of tentacles; spermathecal duct extends to anterior end of mantle cavity.
8. Seminal receptacle joins common sperm duct (illustrated in Davis, 1980, and Davis & Greer, 1980).
9. Specialized shell shape, specialized central tooth, unique female reproductive system (see Davis, 1979; Davis, 1980; Davis & Greer, 1980).

(Davis, 1979, 1980). It is possible that lineages B_1 and B_2 derived from different marine ancestors of rissoacean grade organization.

DISCUSSION

Higher category relationships

There are still insufficient data for hydrobioids world-wide to attempt a definitive treatment such as that by Radoman (1973), who created the superfamily Hydrobioidea with nine families and eleven subfamilies while considering only eastern European taxa. Hydrobioids are defined as an artificial grouping of fresh and brackish water snails that resemble each other in generalized shell, opercular, radular, and penial characters seen in taxa considered Hydrobiidae pre-1979 (Davis, 1979, 1980).

We need to assess the cladistic relationships among hydrobioids through a careful analysis of characters and their states, geological and paleontological records, zoogeographic patterns, and ecological factors affecting morphological character-states. In this paper we restrict higher category relationships to discussing the Hydrobiidae with subfamilies Hydrobiinae, Lithoglyphinae, and Nymphophilinae. The Pomatiopsidae with its subfamilies, Pomatiopsinae and Triculinae, have been fully discussed elsewhere (Davis, 1979, 1980).

Potamolithus does not have the morphology of the Pomatiopsidae. Further, no hydrobioid from Africa, India, Southeast Asia (including Southwest China) has, to our knowledge, *Potamolithus*-like morphology. *Potamolithus* shares more presumably derived character-states with *Lithoglyphus* of Europe than with any other taxon for which we have anatomical data and is therefore classified as Hydrobiidae: Lithoglyphinae. We consider the character-state differences separating *Lithoglyphus naticoides* and *P. ribeirensis* to justify generic status but realize that more species of *Potamolithus* must be studied to determine the character-states shared among them. For example, is the nuchal node common to all species of *Potamolithus*? Is the pleurosupraesophageal connective relatively long in all species, etc.? The concentrated nervous system of *Lithoglyphus*, based on data for *L. naticoides* is not sufficient reason, of itself, to place *Lithoglyphus* in a separate subfamily or family as has been done (Radoman, 1973). It is also necessary to study *L. apertus* Küster and *L. fuscus* Pfeiffer in detail over the broad suite of characters presented here, to better define the genus *Lithoglyphus*.

Convergence and defining higher taxa

Convergence is probably the most underestimated phenomenon in molluscan systematics (Davis, 1979). Unless it is detected and isolated, assessments of relationships will be in error (Cain & Harrison, 1960). Some thirty species and subspecies of *Potamolithus* have been monographed (Pilsbry, 1911). The shells of these taxa are of a size and shape variation such that many of the species resemble species of the pomatiopsid genera *Lacunopsis* and *Jullienia* of the Mekong River in Southeast Asia (Brandt, 1974; Davis, 1979). The squat head and penis morphology of *Lithoglyphus lapidum* (= *P. ribeirensis*) (von Ihering, 1895) likewise indicate a possible close cladistic relationship between *Potamolithus* and the Mekong River taxa. It is these similarities that prompted this study.

It is clear that these hydrobioid radiations converge. With the aid of multivariate analysis, several character-states correlate with snails having a globose to cap-shaped shell, i.e. squat head, powerful wide foot, etc., characters presented earlier. The shell size, shape and correlated character-states are associated with ecological factors, namely living in a high energy environment, clinging to rocks and boulders. Accordingly, such convergent characters should not be used to define clades, or if used, it should be with caution. It should be noted that there are species with globose shells living in other microhabitats such as on aquatic vegetation or plowing along on a sandy mud substrate. Many of these are small Hydrobiidae: Amnicolinae with shell lengths <4.0 mm; others are Hydrobiidae: Nymphophilinae. The ovate-conic shell form is generalized while the globose shape is specialized (Davis, 1979, 1980). As with the X^2 analysis of the relationship of specialized character-states with degrees of current for species in the Mekong River (Davis, 1979, p. 73), it appears that there is a lack of species with generalized states living in swift current and not the absence of species with specialized states from slow water environments.

It is evident from the ordination diagrams that although *Potamolithus*, *Lithoglyphus*, and *Lacunopsis* converge in seven character-states, they are highly divergent overall. It is also evident that *Potamolithus* and *Lithoglyphus* sharing a set quite removed from *Lacunopsis*, are more closely associated to each other than *Lacunopsis* is to other taxa of the Pomatiopsidae. *Lacunopsis*, of all the taxa represented has the most specialized character-states (Davis, 1979; 1980; Davis & Greer, 1980).

The Pomatiopsidae and their subfamilies Pomatiopsinae and Triculinae have been defined (Davis, 1979, 1980). The study of Triculinae indicates a considerable range of shell shape and sculpture patterns within a subfamily, tribe and genus. For example, in the genus *Lacunopsis*, shapes range from ovate-globose, to globose, neritiform, and *Calyptraea*-form (Davis, 1979). We have discussed above how certain suites of characters can correlate with shell shape. Osphradial length in some instances appears associated with ecological factors (Davis, 1979). It follows, therefore, that one must beware of defining higher taxa by scoring taxa on the basis of similarity of character-states without consideration of their adaptive significance.

It has been argued that genera are not simply artificial groupings of species (Davis, 1981). Adaptive radiation studies indicate that one or more novel innovations, morphological or physiological, may be associated with a new adaptive zone. Success in that zone may result in speciation with species occupying different niches. Morphological or physiological changes associated with different niche dimensions serve to define the species while the features, common to all species of the radiation, that are associated with the new adaptive zone serve for recognition of the genus. Thus defined, a genus is a first order radiation of an adaptive radiation (Davis, 1981).

In this regard, the concentrated nervous system and blunt penis type without eversible papilla, together with a generalized type of ciliated sperm groove of the pallial oviduct, apparently serve to define *Lithoglyphus*. The generalized, more open nervous system, more slender penis with eversible papilla, innovative folded ciliated sperm groove and bolster, plus nuchal node may (if more species have these features) serve to define *Potamolithus*. The character-states that combine these genera in the same subfamily are: 1) the lack of a fold and protuberance of the stomach's posterior chamber; 2) there are two or more pairs of basal cusps on the central tooth; one or more innermost pairs of basal cusps arise from the face of the central tooth, not the lateral angle; 3) the penis is simple, i.e. with one duct and without lobes or special complex glandular protuberances; 4)

eggs are not covered in a case covered with sand grains; 5) the shell is globose, with correlated morphological character-states.

The last character-state is, in light of the preceding discussion, the weakest because we have emphasized the potential for shell shape variation within a genus, tribe, and subfamily. Thus far, the globose shell is associated with the other four character-states serving to define the Lithoglyphinae. We will maintain this character-state for the subfamily until such time that a hydrobioid species with turreted or ovate-conic shell is found that also has the other four character-states of the Lithoglyphinae.

The Hydrobiinae include those genera that have: 1) a fold and protuberance of the stomach's posterior chamber; 2) one or more pairs of basal cusps arising from the lateral angle, but none from the face of the tooth (Davis, 1979); 3) penis simple, with one duct, with or without small glandular appendage on the concave curvature; 4) eggs with or without sand covering; 5) shell shape variable. Stomach morphology is not known for numerous key taxa, so the use of the fold and protuberance (so-called appendix) character-state introduced by Radoman (reviewed by Radoman, 1973) has yet to be fully evaluated. The presence of one or two seminal receptacles, the loss of seminal receptacles with sperm stored in the coil of the oviduct, or the storage of sperm in secondary seminal receptacles are considered possible within a single subfamily (see Davis, 1979; Thompson, 1979).

Some of the character-states listed in Table 9 are diagnostic for the genus *Hydrobia* and do not serve to define the Hydrobiinae. These are the pallial tentacle, hypertrophied cilia on the left tentacle, a single pair of basal cusps on the central tooth of the radula, and a simple glandular lobe of the penis in most species. The first two character-states are most probably associated with life in marine or brackish water. Given these genera-specific diagnostic features, there is much less divergence in ground-plan between the Hydrobiinae and Lithoglyphinae of the Hydrobiidae than between the Pomatiopsinae and Triculinae of the Pomatiopsidae.

Thompson (1977, 1979) presented data for *Marstonia* of the United States and *Nymphophilus* of Mexico, respectively. He argued that these and allied genera were monophyletic and belonged to the Hydrobiidae: Nymphophilinae, of which Radoman's (1973) Orientaliidae were synonymous. Of the nine character-states presented by him, all but two are those pertaining to the family diagnosis or diagnosis for the Hydrobiinae given above. The two truly diagnostic character-states are 1) the bilobed nature of the penis and, 2) various complex raised glandular structures called apocrine glands. Taxa with these nine character-states are found in Europe as well as North America. In addition to the nine listed character-states, the genera of the Nymphophilinae have similar embryonic shell microsculpture different from that of other North American hydrobioid taxa (Thompson, 1979). If the embryonic shell sculpture character-state is consistently valid, and if the stomachs of these genera lack the fold and protuberance seen in the Hydrobiinae, then there would perhaps be sufficient character-state divergence from the Hydrobiinae to justify subfamilial status. The Orientaliidae of Radoman lack the stomach fold and protuberance, as do the Lithoglyphinae.

Zoogeographic relationships

Parodiz (1969) stated that there were three possible hypotheses to account for the origin of *Potamolithus*: 1) the group is ancient, cosmopolitan, derived from Gondwanaland; 2) the genus dispersed to South America from ancestors of North American origin; 3) the genus is modern, derived from marine ancestors. Parodiz discounted the Gondwanaland origin because of the scant fossil record, i.e. a few Paleocene and lower Eocene occurrences in Chile and Argentina with no other records until the Recent. Parodiz favored the third hypothesis.

Pilsbry (1911) wrote a definitive essay on the origins of non-marine molluscan faunas of South America. He considered *Potamolithus* to have an Austral-South American origin and stated that nothing in the distribution of mollusks would lead to the hypothesis that South Africa had ever been connected with Antarctica and thereby indirectly with southern South America. He noted that *Fluminicola* of western North America, *Lithoglyphus* of Europe, *Pachydrobia* [*sensu lato, non* Davis, 1979] *Lacunopsis* and *Jullienia* of Indo-China had historically been grouped together in the Lithoglyphinae because of similarities of shell shape. However, he considered *Potamolithus* to be most closely related to *Petterdiana* within the Amnicolidae: Amnicolinae, the latter genus from Tasmania-Australia. The other

above-mentioned genera belonged to the Amnicolidae: Lithoglyphinae [Amnicolidae = Hydrobiidae of present usage]. The character used to differentiate the two subfamilies was the number of cusps on the marginal teeth: few and large = Lithoglyphinae; many (>20) and small = Amnicolinae. Pilsbry (1911) stated that, as he found the number of basal cusps so variable in many genera, he did not attach much importance to that character.

Pilsbry (1911) found the Austral-South American generalized track in part from the individual tracks of *Potamolithus*, the *Potamolithus-Petterdiana* connection, and the freshwater clam *Diplodon*. He noted that *Potamolithus* was lacking from India, Southeast Asia, or the Far East.

Pilsbry (1911) was too hasty in discounting an African connection for faunal elements in common between South America and Australia or between Africa and southern South America. Of particular importance are the corneous operculated Ampulariidae, Planorbidae, and Mutelidae that have closely related taxa in South America and Africa with those elements reaching North America (Mexico, Florida) by dispersal from the south.

The pomatiopsid *Aquidauania*[4] of southwestern Brasil is closest in relationships with South African *Tomichia* and Australia *Coxiella* (Davis, 1979, 1980). *Potamopyrgus* has been reported from Africa (Brown, 1980), but this African taxon must be studied anatomically; it may not be *Potamopyrgus*. Additionally, the African genera *Lobogenes* and *Soapitia* that are considered Hydrobiidae (Brown, 1980) must be examined as certain shell features suggest a relationship to *Potamolithus*. In summary, there is a Gondwanaland freshwater component shared between Africa and South America. While no snail with a Lithoglyphinae-type morphology has been found in Africa, one must have data for *Lobogenes* and *Soapitia* to be sure that no named African taxa have such morphology.

There are possible relationships with North American taxa made more probable because of the morphological similarity between *Lithoglyphus* (Fig. 20C) and *Potamolithus*. Strong candidates for cladistic association are *Fluminicola* (Fig. 20B), *Somatogyrus* (Fig. 20A) from southeastern U.S.A., and *Gillia* from eastern U.S.A. The remarkable similarity among shell types of *Potamolithus*, *Lacunopsis*, *Lithoglyphus*, *Fluminicola*, and *Somatogyrus* is seen by comparing Figs. 3, 4, and 21. Of the North American genera the radula and penis type shown or discussed for *Gillia* (Walker, 1918; Stimpson, 1865; and *Somatogyrus* (based on *S. tenax*, Thompson, 1969) come the closest to *Potamolithus*. Presence of snails with the female reproductive system morphology of the Hydrobiidae, exclusively in freshwater systems of North America, has been demonstrated, i.e. *Marstonia* and *Nymphophylus* (Thompson, 1977, 1979). We predict that the placement of *Fluminicola, Gillia,* and *Somatogyrus depressa* (Tryon) (the type-species) in the Hydrobiidae based on total morphology, will be justified. Stimpson (1865) followed by Walker (1918) illustrated and/or discussed the penis of *Somatogyrus* to be broad and bifid. This description was based on *Melania isogona* Say. Baker (1926, 1928) pointed out that the penis of *Somatogyrus* is simple and not bifid. He erected the genus *Birgella* for taxa with the penis and radula of *Melania isogona*. The type-species is *B. subglobosa* (Say) and *M. isogona* was relegated to *B. subglobosa isogona*. *Birgella* is included in the Hydrobiidae Nymphophilinae (Thompson, 1979).

In summary of zoogeographic relationships this study proves conclusively the presence of genuine Hydrobiidae in South America. Morphological evidence conclusively proving the presence of the Hydrobiidae in southern continents had previously been lacking (Davis, 1979, 1980). We reject Parodiz's third hypothesis. Given the antiquity of hydrobioids (Permian of Africa, Knight et al., 1960), their widespread distribution, and considering the Paleocene-Eocene records from South America, *Potamolithus* most likely did not evolve from marine ancestors in the late Tertiary, and certainly not in the Recent. The *Potamolithus-Petterdiana* hypothesis of relationship must be proven, but if correct, it would demonstrate an old Austral-South American relationship. The close morphological relationship to *Lithoglyphus* of Europe, the character of marginal cusp number notwithstanding, probably indicates divergence of the Lithoglyphinae before the breakup of Pangaea. The vicariance-dispersal patterns affecting the modern distribution of the Litho-

[4]Malek (1983) has shown that *Aquidauania* Davis, 1979 is a synonym of *Idiopyrgus* Pilsbry, 1911 by presenting anatomical data for the type-species of *Idiopyrgus*, i.e. *I. souleyetianus* Pilsbry, 1911. This clarification was made after this paper was in press.

glyphinae cannot, however, be ascertained without detailed morphological data for the American, African, and Australia-Tasmanian taxa discussed above, as well as for numerous other taxa in Mexico, Central and South America about which we know nothing except for shell and radula data. American taxa for which we have few data are among those listed in Taylor (1966) and Thompson (1968).

ACKNOWLEDGEMENTS

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LITERATURE CITED

BAKER, F. C., 1926, Nomenclatorial notes on American fresh water Mollusca. *Transactions of the Wisconsin Academy of Science, Arts, and Letters,* 22: 193–205.

BAKER, F. C., 1928, The Freshwater Mollusca of Wisconsin pt. 1. Gastropoda. *Bulletin of the Wisconsin Geological and Natural History Survey* No. 70, Madison, IL, xx + 507 p., 28 pl.

BRANDT, R. A. M., 1974, The non-marine aquatic Mollusca of Thailand. *Archiv für Molluskenkunde,* 105: 1–423.

BROWN, D. S., 1980, *Freshwater Snails of Africa and Their Medical Importance.* Taylor & Francis, London, x, 1–487 p.

CAIN, A. J., 1964, The perfection of animals. In: CARTHY, J. E. & DUDDINGTON, C. L., eds., *Viewpoints in Biology* No. 3, p. 36–63, Butterworths, London.

CAIN, A. J. & HARRISON, G. A., 1960, Phyletic weighting. *Proceedings of the Zoological Society of London*, 136: 1–31.

DAVIS, G. M., 1979, The origin and evolution of the Pomatiopsidae, with emphasis on the Mekong River Triculinae. *Monograph of the Academy of Natural Sciences of Philadelphia,* No. 20: i–viii, 1–120.

DAVIS, G. M., 1980, Snail hosts of Asian *Schistosoma* infecting man: evolution and co-evolution. In: BRUCE, J. & SORNMANI, S., eds., *The Mekong Schistosome, Malacological Review Supplement* No. 2, p. 195–328.

DAVIS, G. M., 1981, Different modes of evolution and adaptive radiation in the Pomatiopsidae (Prosobranchia: Mesogastropoda). *Malacologia*, 21: 209–262.

DAVIS, G. M. & CARNEY, W. P., 1973, Description of *Oncomelania hupensis lindoensis*, first intermediate host of *Schistosoma japonicum* in Sulawesi (Celebes). *Proceedings of the Academy of Natural Sciences of Philadelphia,* 125: 1–34.

DAVIS, G. M. & GREER, F. J., 1980, A new genus and two new species of Triculinae (Gastropoda: Prosobranchia) and the transmission of a Malaysian mammalian *Schistosoma* sp. *Proceedings of the Academy of Natural Sciences of Philadelphia,* 132: 245–276.

DAVIS, G. M., KITIKOON, V. & TEMCHAROEN, P., 1976, *"Lithoglyphopsis" aperta* (Gastropoda): morphology, evolutionary relationships, and the transmission of Mekong schistosomiasis. *Malacologia,* 15: 241–287.

HERSHLER, R. & DAVIS, G. M., 1980, The morphology of *Hydrobia truncata* (Gastropoda: Hydrobiidae): relevance to systematics of *Hydrobia. Biological Bulletin,* 158: 195–219.

HOAGLAND, K. E. & DAVIS, G. M., 1979, The stenothyrid radiation of the Mekong River. 1. The *Stenothyra mcmulleni* complex (Gastropoda: Prosobranchia). *Proceedings of the Academy of Natural Sciences of Philadelphia,* 131: 191–230.

IHERING, H. VON, 1885, Zur Kenntniss der Gattung *Lithoglyphus. Malakozoologische Blätter,* new ser., 7: 96–99, 1 pl.

KNIGHT, J. B., BATTEN, R. L., YOCHELSON, E. L. & COX, L. R., 1960, *in* MOORE, R. C., ed., *Treatise on Invertebrate Paleontology.* Part I. Mollusca I: 310–324. University of Kansas Press, Lawrence.

KRAUSE, H., 1949, Untersuchungen zur Anatomie und Ökologie von *Lithoglyphus naticoides* (C. Pfeiffer). *Archiv für Molluskenkunde*, 78: 103–148.

KRULL, A., 1935, Anatomische Untersuchungen an einheimischen Prosobranchiern und Beiträge zur Phylogenie der Gastropoden. *Zoologische Jahrbucher (Anatomie)*, 60: 399–464.

MALEK, E. A., 1983, The South American hydrobioid genus *Idiopyrgus* Pilsbry, 1911. *Nautilus*, 97(1): 16–20.

PARODIZ, J., 1969, The Tertiary non-marine Mollusca of South America. *Annals of Carnegie Museum,* 40: 1–242.

PILSBRY, H. A., 1896, Notes on new species of Amnicolidae collected by Dr. Rush in Uruguay. *Nautilus*, 10: 86–90.

PILSBRY, H. A., 1911, Non-marine Mollusca of Patagonia. In: SCOTT, W. B., ed., *Reports of the Princeton University Expeditions to Patagonia, 1896–1899,* 3(2), pt. 5: 513–633.

RADOMAN, P., 1966, The zoogeographical and phylogenetic interrelationships of the genera *Lithoglyphus* and *Emmericia. Bulletin du Museum d'Histoire Naturelle, Belgrade,* ser. B, 21: 43–49.

RADOMAN, P., 1973, New classification of fresh and bra[c]kish water Prosobranchia from the Balkans and Asia Minor. *Prirodnjački Muzej u Beogradu. Posebna Izdanja.* Éditions hors série Knjiga, 32: 1–30.

RADOMAN, P., 1976, Hydrobiidae auf der Balkanhalbinsel und Kleinasien. *Archiv für Molluskenkunde*, 107: 203–223.

ROLF, F. J., KISHPAUGH, J. & KIRK, D., 1972, NT-SYS: Numerical taxonomy system of multivariate statistical programs. Technical Report, State University of New York, Stony Brook, N.Y.

STIMPSON, W., 1865, Researches upon the Hydrobiinae and allied forms; chiefly made upon materials of the Smithsonian Institution. *Smithsonian Miscellaneous Collections,* 201: 1–59.

THOMPSON, F. G., 1968, *The aquatic snails of the family Hydrobiidae of Peninsular Florida.* University of Florida Press, Gainesville, 268 p.

THOMPSON, F. G., 1977, The hydrobiid snail genus *Marstonia. Bulletin of the Florida State Museum, Biological Sciences,* 21: 113–158.

THOMPSON, F. G., 1979, The systematic relationships of the hydrobioid snail *Nymphophilus* Taylor 1966 and the status of the subfamily Nymphophilinae. *Malacological Review,* 12: 41–49.

WALKER, B., 1918, A synopsis of the classification of the fresh-water Mollusca of North America, North of Mexico. *Miscellaneous Publications of the University of Michigan Museum of Zoology,* No. 6: 1–213.

WILSON, E. O., 1965, A consistency test for phylogenies based on contemporaneous species. *Systematic Zoology,* 14: 214–220.

APPENDIX:
The Identity of *Potamolithus ribeirensis* Pilsbry

The description of *Potamolithus ribeirensis* Pilsbry, 1911 was based on six shells from Brasil, São Paulo State, Ribeira River, Yporanga (= Iporanga) collected by von Ihering in 1908 (Fig. 1). The syntypes had ANSP catalog number 103076. One of us (Davis) selected a lectotype (Fig. 3A; ANSP 103076) and recataloged the remainder as paralectotypes (ANSP 353441). The species was described as having 3.5 convex whorls, the last globose; length 3.5 mm, width 3.4 mm, and length of aperture 2.7 mm. The species differed from *P. lapidum* by its broad columella and small size.

The shells from the Feitoria river some 800 km SSW of the Ribeira River closely resemble those of the type-series except that they have as adults, 4.0 to 4.5 whorls and are larger. Statistics of shell parameters between the populations can be compared by examining Tables 2 and 3.

The populations were compared using the multivariate analysis program NT-SYS (Rohlf *et al.,* 1972). The six syntypes and ten individuals from the Feitoria River (chosen at random from the most common size classes of 4.0 and 4.5 whorls) were compared over eight shell characters (Table 13). Distance coefficients were generated using UPGMA. We used the minimum spanning tree (MST) and subsets components. Character correlations were subjected to Principal Component Analysis (PCA) with components extracted until eigenvalues became less than 1.0. A transposed matrix of the first three principal components was used to produce a matrix of OTU projections in the principal component space. The resulting PCA-based configuration was used as the initial configuration for nonmetric multidimensional scaling (MDS). A cophenetic correlation was calculated by comparing the distances between OTUs in the PCA- and MDS-spaces and the Q-mode taxonomic distance matrix. The ordination diagram based on MDS is freed from the constraints of phenogram construction.

The results are shown in Figs. 21 and 22. The cophenetic correlation for the phenogram based on three-dimensional scaling was 0.81; the cophenetic correlation for MDS $\times$ the Q-mode taxonomic distance matrix was 0.99. In PCA the first component accounted for 83.7% of the variance; the second component 11.95%; the third, 1.74%. Eigenvalues were: first component, 6.69; second component, 0.96. All but character 7 loaded highly on the first axis (values $>$ 0.879). It is evident, as discussed below, that character 7 (loading 0.439 on axis 1) loaded highly on axis 2 of Fig. 22. The stress for three-dimensional scaling was 0.009. It is clear from the phenogram and ordination diagram that the individuals of the two populations do not separate into discrete clusters. As expected, individuals separate along the first axis on the basis of size, largest to the far left, smallest to the far right. Only the extreme whorl numbers follow this pattern, i.e. 4.5 whorls to the far left; 3.0 whorls, far right (no. 3). Individuals with 3.5 or 4.0 whorls are not separated in order along axis 1. Separation along axis 2 is clearly based on width or columellar callus, the more slender below axis 1; the wider, above axis 1. The lectotype (no. 1) is close to the center of the diagram. Minimum spanning tree (Prim Network) and subsets in relationship to the

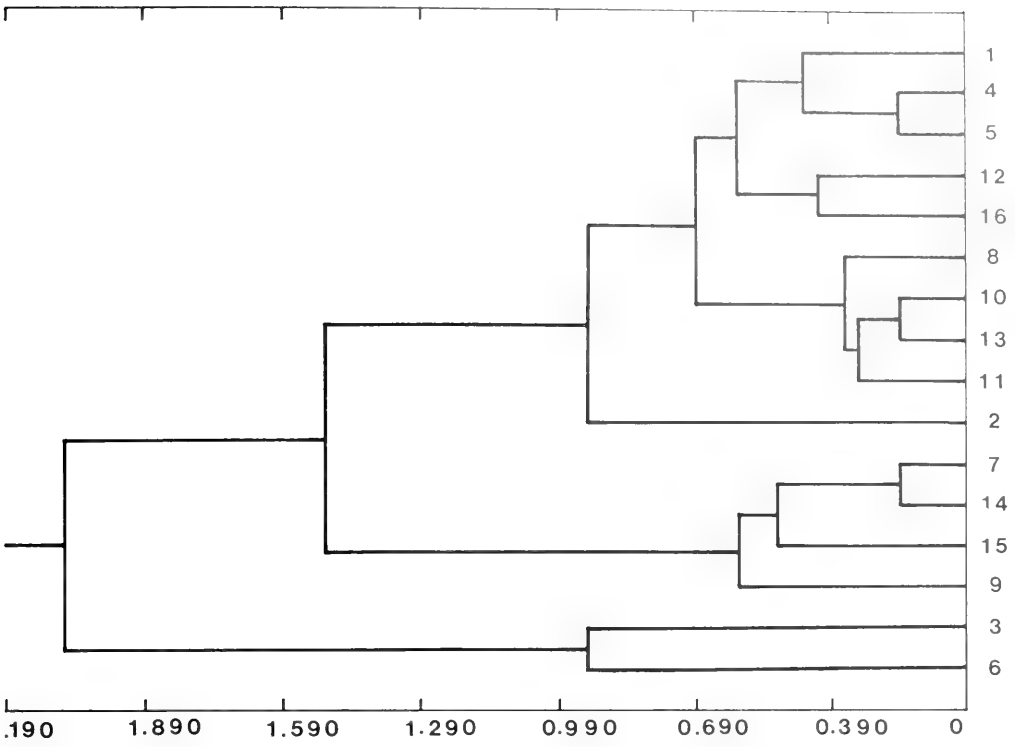

FIG. 21. Phenogram showing phenetic relationships among 16 individuals from the type-series and from our study site (see Table 13 and Appendix).

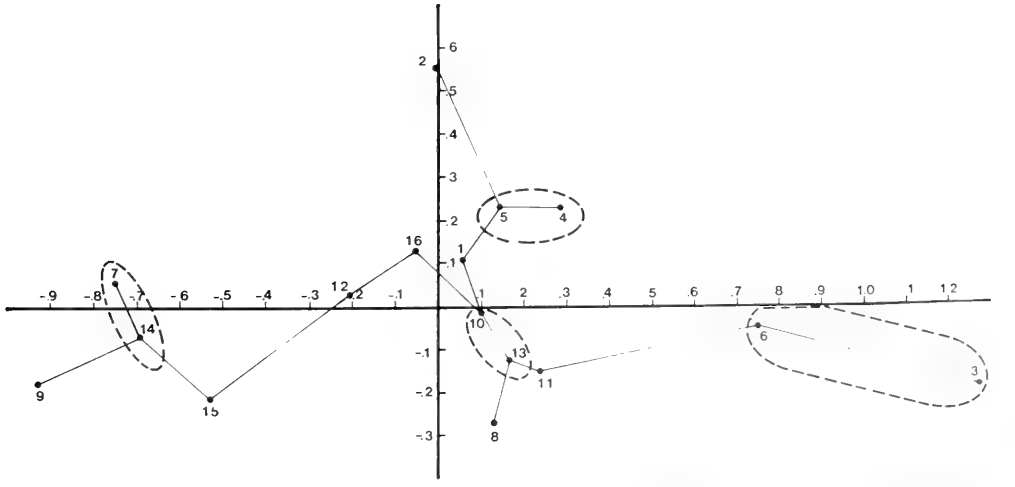

FIG. 22. Ordination diagram following three-dimensional scaling, showing position of 16 individual shells along axes 1 and 2. Dotted lines are sets. See Table 13 and Appendix.

TABLE 13. Matrix of sixteen OTUs and eight shell characters to facilitate a comparison of the type-population with the Feitoria River population using multivariate analyses. Measurements in mm.

	Shell character							
OTU	1 Whorl no.	2 Shell length	3 Length body whorl	4 Width	5 Length aperture	6 Width aperture	7 Width columellar callus	8 Width penultimate whorl
					Ribeira River			
1 Lectotype	3.5	3.68	3.40	3.36	2.92	2.44	0.48	1.0
2 Paralectotype	3.5	3.68	3.32	3.32	2.96	2.48	0.60	1.0
3 Paralectotype	3.0	2.28	2.04	2.28	1.88	1.60	0.36	0.64
4 Paralectotype	3.5	3.32	2.96	3.04	2.68	2.24	0.52	1.0
5 Paralectotype	3.5	3.64	3.24	3.20	2.68	2.32	0.52	1.04
6 Paralectotype	3.5	2.84	2.56	2.60	2.24	1.88	0.44	0.76
					Feitoria River			
7	4.5	4.64	4.08	4.04	3.44	2.96	0.52	1.28
8	4.0	3.68	3.28	3.08	2.80	2.20	0.40	1.08
9	4.5	5.00	4.40	4.24	3.12	3.24	0.48	1.44
10	4.0	3.60	3.20	3.20	2.84	2.20	0.48	1.0
11	4.0	3.40	2.96	2.96	2.56	2.24	0.44	1.12
12	4.0	3.92	3.52	3.60	3.16	2.60	0.48	1.08
13	4.0	3.52	3.12	3.12	2.72	2.28	0.44	1.0
14	4.5	4.60	4.00	4.00	3.40	3.00	0.48	1.28
15	4.5	4.36	3.80	3.88	3.20	3.00	0.44	1.20
16	4.0	3.68	3.24	3.36	2.80	2.48	0.52	1.12

distribution of OTUs in the diagram indicate that a single species is involved where differences among individuals involve size differences and width of columellar callus. Individuals of the type-population apparently reach maximum size at 3.5 whorls and have an average length at that whorl stage of 3.44 ± 0.37 mm (n = 5). One third (33%) of the individuals have a relatively slender columellar callus. Individuals of the other population reach 4.5 whorls with an average length of 4.65 mm ± 0.26 mm (n = 4) but have predominantly 4.0 whorls with an average length of 3.46 ± 0.43 (n = 50) and about 70% have a relatively slender columellar callus. Because the Feitoria population matures at 4.0 to 4.5 whorls and has a generally more slender columellar callus we distinguish this population as race-B contrasted with the type-population as race-A. Pilsbry (1911) figured one shell (plate XLIb, fig. 4) from a series collected by von Ihering from the Ribeira River but from a locality called Hiririea; this shell (Fig. 4A) has a "wide, lunate, concave umbilical area, defined by an acute black keel, the columella as wide, as in *P. ribeirensis*" (ANSP 103068). This specimen is eroded but undoubtedly of 4.0 to 4.5 whorls. The shell is 4.2 mm long. The periostracum has a greenish color. The large size indicates that some populations in the Ribeira River may reach maturity at 4.0 to 4.5 whorls and have a size similar to that of race-B. This must be determined. The strong keel seen in some individuals is not unusual, as discussed in the body of this paper.

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NORTH AMERICAN FRESHWATER SNAIL GENERA OF THE HYDROBIID SUBFAMILY LITHOGLYPHINAE

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ABSTRACT

The classification of the North American genera of Lithoglyphinae is reviewed, based on anatomical and conchological characters. Five genera are recognized: *Gillia* Stimpson, 1865, *Fluminicola* Stimpson, 1865, *Somatogyrus* Gill, 1863, *Clappia* Walker, 1909, and *Lepyrium* Dall, 1896. The North American genera are conservative in their anatomies. Primary morphological differentiations involve radular and shell characters. The North American genera are demonstrated to have evolved through trophic specializations and microhabitat specializations.

Lepyrium Dall, 1896, formerly considered a monotypic family, is closely related to *Clappia* and *Somatogyrus*. *Birgella* Baker, 1926, is removed from the Lithoglyphinae, where it has been placed by authors, and is demonstrated to be in the Nymphophilinae. The following species are described in detail: *Lepyrium showalteri* (Lea, 1861), *Somatogyrus rheophilus* n. sp., *Gillia altilis* (Lea, 1841). A neotype is designated for the latter species.

Key words: Gastropoda, snails, Hydrobiidae, Lepyriidae, Lithoglyphinae, *Lepyrium, Clappia, Somatogyrus, Gillia, Fluminicola,* systematics, evolution.

INTRODUCTION

This paper discusses the systematic relationships between the North American lithoglyphine genera: *Lepyrium, Somatogyrus, Clappia, Gillia,* and *Fluminicola* (Class GASTROPODA, Subclass PROSOBRANCHIA, Order MESOGASTROPODA, Family HYDROBIIDAE, Subfamily LITHOGLYPHINAE). A sixth genus, *Antrobia* Hubricht, 1972, is placed by Burch & Tottenham (1980: 100) in the Lithoglyphinae. It is a monotypic genus from a cave in Missouri; the anatomy remains undescribed and *Antrobia* is omitted from further discussion in this paper. Another genus, *Cochliopina* Morrison, 1946, traditionally has been associated with the Lithoglyphinae. Hershler (in press) shows that it is a genus of the Littoridininae. *Birgella* Baker, 1926, is another genus that has been confused with this subfamily, even as recently as 1981 (Clarke). It is in the NYMPHOPHILINAE, as is discussed in Appendix B.

This study stems from two independent investigations. The first was an attempt to determine species-group characteristics within *Somatogyrus*, a genus containing many species (Burch & Tottenham, 1980: 104–106). The study was tabled temporarily because very little anatomical diversity was discovered among the species examined. Independently I examined the anatomy of *Lepyrium showalteri* (Lea), a snail previously placed in a monotypic family of uncertain affinity within the MESOGASTROPODA (Pilsbry & Olsson, 1951). Its soft anatomy was found to be hardly distinguishable from that of *Somatogyrus*. These two genera have very dissimilar shells, but they have in common similar habitats. *Lepyrium* and most *Somatogyrus* live on rocks and boulders in high-energy rivers. The habitat deployments among these two genera focus on the adaptive radiation of the Lithoglyphinae in eastern North America. In order to clarify the limits of this basic radiation other relevant genera were examined. The results of these studies are presented herein.

MATERIAL AND METHODS

Anatomical descriptions and illustrations in this paper are based upon the following specimens:

Lepyrium showalteri (Lea). Two lots of about 100 specimens each, collected June 21, 1978 (UF 31343) and June 22, 1978 (UF 31342) in the Little Cahaba River, 2.4 km upstream from the Cahaba River, Bibb Co., Alabama by F.G.T. Relaxed with menthol crystals, fixed in Bouin's solution and preserved in 70% ethanol.

Somatogyrus rheophilus n. sp. (described below). One series of thousands of speci-

mens collected October 21, 1973, in the Flint River, 9.7 km SW of Lincoln Park, Upson Co., Georgia by F.G.T. (UF 40511). Relaxed with menthol crystals, fixed in Bouin's solution and preserved in 70% ethanol.

Gillia altilis (Lea). One series of about 400 specimens collected June 1, 1980 in Lake Waccamaw, Columbus Co., North Carolina by Hugh J. Porter (UF 27550). Fixed unrelaxed and preserved in 70% ethanol.

Fluminicola nuttalliana (Lea). One series of 23 specimen collected July 20, 1974 in the Satsop River at Satsop, Grays Harbour Co., Washington by Dennis R. Paulson (UF 34813). Fixed unrelaxed and preserved in 70% ethanol.

Dissections were made in 70% ethanol under a WILD M-2 dissecting microscope. Serial sections were made at 10 μm and stained in 10% Harris' hematoxylin stain. Radulae were cleaned in a saturated solution of potassium hydroxide and examined with a HITACHI S-415A scanning electron microscope at the Department of Zoology, University of Florida.

Museum abbreviations are given in Appendix C.

ANATOMY OF THE GENERA

The genera *Lepyrium, Clappia, Somatogyrus, Gillia* and *Fluminicola* are placed in the subfamily LITHOGLYPHINAE. The conical, or depressed-conical, or globose-conical, or neritoid-shaped shells have in common spiral sculpture on the protoconch (Figs. 39–42). Other protoconch sculpture may also be present. Other aspects of the shell are not distinctive at the subfamily levels among North American hydrobiid genera, although the lithoglyphines tend to be stocky species with relatively heavy shells.

A high degree of anatomical uniformity exists among the North American LITHOGLYPHINAE. Principal morphological differences occur in trophic structures and in the shell. Minor diversity also occurs in the male and female reproductive systems. Because of this basic uniformity it is convenient to describe the anatomy of *Lepyrium* and compare other genera with it.

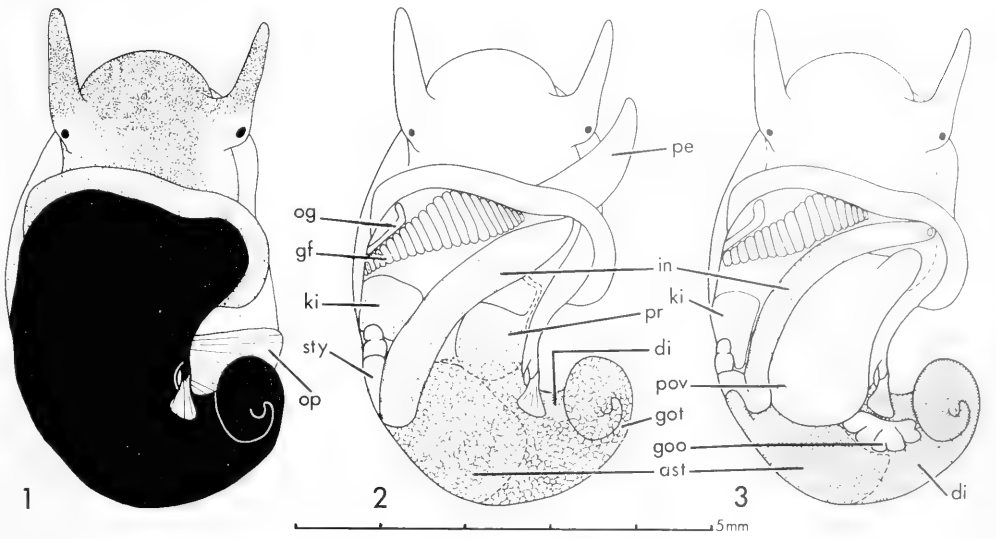
Lepyrium Dall, 1896

Lepyrium is a monotypic genus endemic to the Coosa and Cahaba Rivers in Alabama where it lives on boulders in high energy shoals. *Lepyrium showalteri* (Lea) is described in detail because its systematic affinities have been in question since it was discovered. Its shell is described in Appendix A. The shell (Figs. 59–62) is peculiar because it has a depressed spire and an expanded, flattened body whorl, giving it a neritid appearance. The first whorl of the protoconch is smooth, except for a few short spiral furrows below the periphery (Fig. 39). The neomelanian type operculum (Fig. 7) is modified in shape to conform to the enlarged aperture. This is accomplished by rapid expansion of the last whorl from the paucispiral nucleus, a minor elaboration of the basic condition that also exists in *Somatogyrus* and *Gillia* (Fig. 52). Its radular teeth are modified for feeding on small-sized food particles. *Lepyrium* is the most divergent of the North American LITHOGLYPHINAE because of these specializations. Other aspects of its anatomy are conservative and indicate a close relationship to *Clappia* and *Somatogyrus*.

External morphology and color. Foot broad and rounded (Figs. 1–3). Operculigerous lobe overlaps on each side, and edge of operculum overlaps extended foot on left side and posteriorly. Mucous groove present along anterior edge of foot. Food grooves and epitaenial folds absent on body and snout. Columellar muscle extending into shell for about a quarter whorl. Columella muscle insertion short but wide, extending transversely nearly the complete width of body whorl (in other genera the insertion is narrower). Mantle collar complete around body, without tentacles or papillae. Mantle cavity of males semicircular in saggital section; triangular in females, bounded posteriorly by pallial gonoduct, pericardium and stomach. Gill (gf) consisting of 19–20 lamellae that are arranged in an oblique series on mantle wall. Lamellae triangular in shape (Fig. 8), with greatest height along intestine. Osphradium (og) long, narrow, L-shaped. Excurrent and incurrent siphons absent. Kidney small, broadly quadrangular, overlying posterior left corner of mantle cavity.

Tentacles long and slender in life, and actively beat substrate in alternate strokes as animal moves about. Snout highly extendable and constantly sampling substrate in moving animal.

Mantle jet black (Fig. 1) on all surfaces, completely opaque to all internal morphology. Snout and tentacles dark gray, fading posteriorly and laterally to light gray on sides of body



FIGS. 1-3. *Lepyrium showalteri* (Lea). Fig. 1. Specimen denuded of shell showing pigmentation of mantle. Fig. 2. Male with melanin removed showing internal anatomy. Fig. 3. Female with melanin removed showing internal anatomy. Legend: ast = anterior chambers of stomach; di = digestive gland; gf = gill filaments; goo = ovary; got = testis; in = intestine; ki = kidney; og = osphradium; op = operculum; pe = penis; pov = pallial oviduct; pr = prostate; sty = style sac.

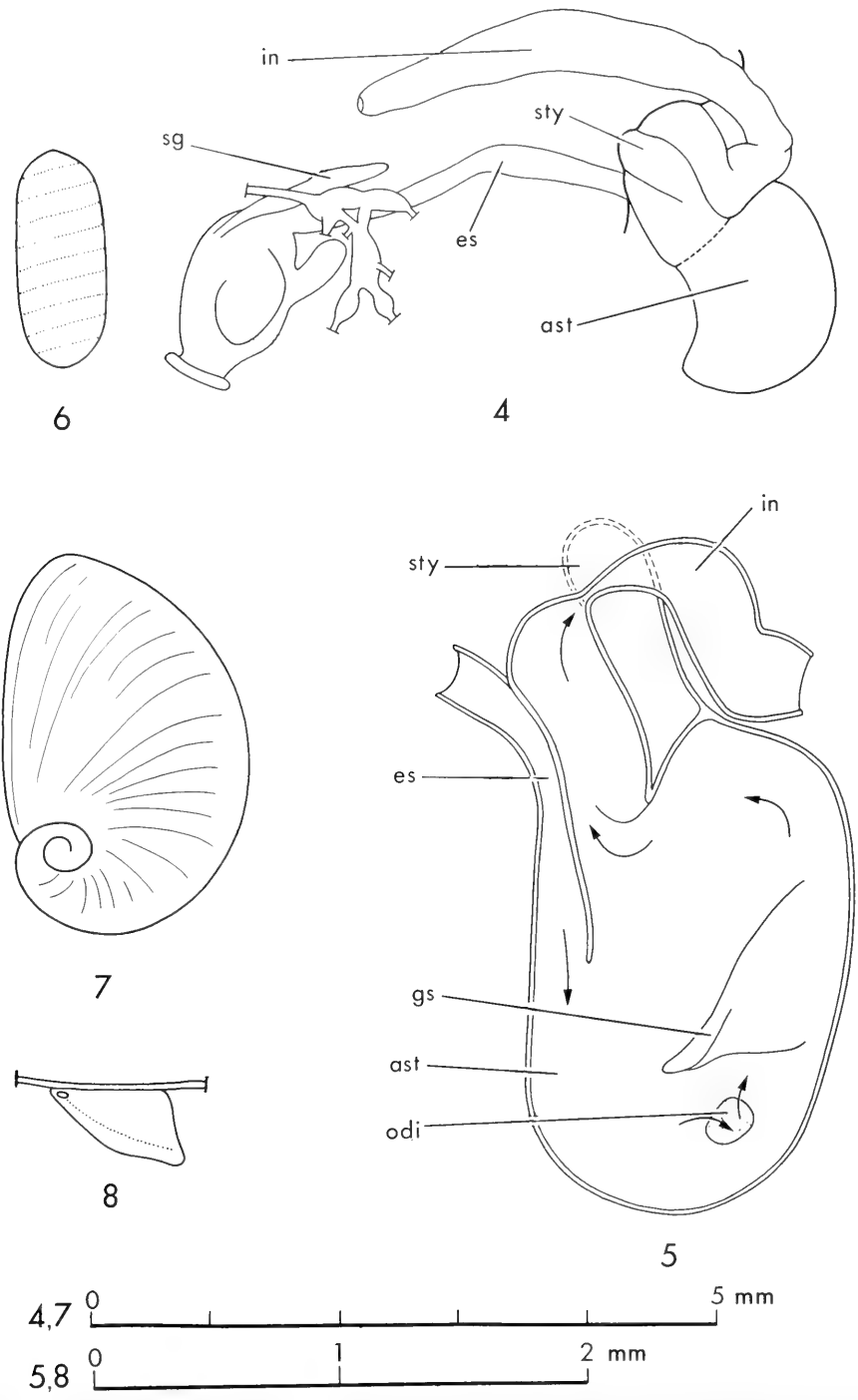
and nape. Tentacles also with diffuse, scattered xanthophores. Sole very light gray. Center of muzzle white. Penis white.

Digestive system. Typically hydrobioid in its configuration (Fig. 4). Two elongate claviform salivary glands (sg) enter posterior buccal mass along dorso-lateral edges, and extend posteriorly over top of nerve ring. Oesophagus (es) entering stomach on left side of posterior chamber (Fig. 5, ast). Stomach with a single opening into digestive gland (odi) posterior to gastric shield (gs). Style sac (sty) slender, at anterior end of stomach on left dorso-lateral surface. Caecae absent. Intestine (in) leaving anterior chamber on left side, passing beneath style sac and up to mantle where it continues diagonally forward to right corner of mantle collar. Anterior end of stomach abutting against kidney, pericardium and pallial gonoduct. In females it is covered by the digestive gland (di) on all sides except anterior end and anterior half of outer wall. In males digestive gland is more restricted due to distribution of testes.

Fecal pellets (Fig. 6) cylindrical, tapered at both ends, and spirally coiled with 4–10 whorls. Pellets about 0.40–0.57 mm long and 0.15–0.20 mm wide. Pellets oblique in intestine at about 45° to longitudinal axis of body.

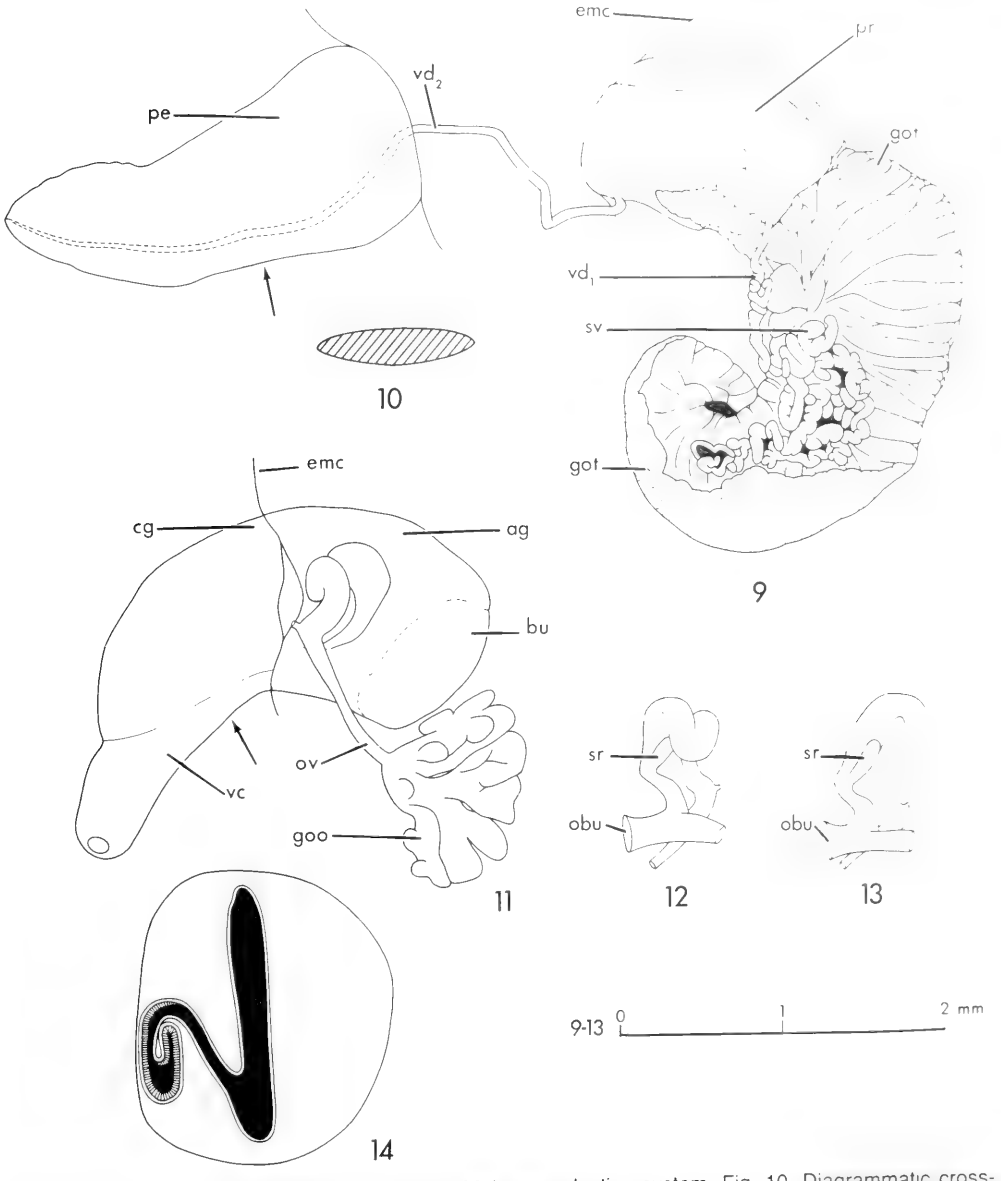
Radula. Taenioglossate (Figs. 15–18, about 1.8 mm long, containing 140–149 transverse rows of teeth (7 specimens examined). Cusps rapidly worn, barely distinguishable on distal third of ribbon. Central tooth (Fig. 15) about 130 μm wide, broadly trapezoidal in shape with a long mid-basal projection; lateral angles with a low ridge bearing 9–11 small, nearly uniform, acuminate basocones on each side (Fig. 16), in contrast to allied genera in which the basocones are enlarged toward the top of the series; dorsal margin weakly reflected with a slightly enlarged mesocone, and 22–24 ectocones on each side. Lateral tooth (Fig. 17) with a long, narrow, laterally projecting, flexed shaft and a strong slender cusp-like basal projection on face of tooth; laterals with 18–22 nearly uniform acuminate cusps. Inner marginals weakly sigmoid with about 50 small cusps. Outer marginals long, slender, with about 35 very small cusps (Fig. 18).

Female reproductive system. (Figs. 3, 11). Ovary (goo) consisting of 3–4 large subequal lobes, each of which is partially divided into smaller lobules. Ovary lying against posterior surface of pallial oviduct (bursa copulatrix) and along right side of stomach, but separated from latter by a thin zone of digestive gland tissue (di); ovary not occupying apical



FIGS. 4–8. *Lepyrium showalteri* (Lea). Fig. 4. Lateral view of digestive system excluding digestive gland. Fig. 5. Ventral view of stomach interior. Fig. 6. Fecal pellet. Fig. 7. Operculum. Fig. 8. Single lamella of gill. Legend: ast = anterior chamber of stomach; es = esophagus; gs = gastric shield; in = intestine; odi = opening to digestive gland; sg = salivary glands; sty = style sac.

whorl of digestive gland. Primary oviduct (ov) relatively stout, passing diagonally forward along ventral side of digestive gland, then mesad to right of oesophagus and style sac and along pericardium at junction of latter with pallial oviduct. A very short gonopericardial duct is present. Primary oviduct enlarging and forming a loop along mesad side of albumen gland; top of loop folded down between rest of loop and albumen gland (Fig. 11). A short



FIGS. 9–14. *Lepyrium showalteri* (Lea). Fig. 9. Male reproductive system. Fig. 10. Diagrammatic cross-section of penis at arrow in 9. Fig. 11. Female reproductive system. Fig. 12. Right side of coil of primary oviduct. Fig. 13. Coil of primary oviduct unfolded to show seminal receptacle. Fig. 14. Cross-section of pallial oviduct at arrow in 11. Legend: ag = albumen gland; bu = bursa copulatrix; cg = capsule gland; emc = posterior wall of mantle cavity; goo = ovary; got = testis; obu = bursa copulatrix duct; ov = oviduct; pe = penis; pr = prostate; sr = seminal receptacle; sv = seminal vesicle; vc = ventral channel; vd_1 = proximal vas deferens; vd_2 = distal vas deferens.

narrow seminal receptacle (sr) projects upward on albumen gland side of descending limb of loop, and is partially covered by the loop so that it can be viewed only by serial section or by teasing the loop free from the albumen gland and unfolding the loop (Figs. 12–13). Seminal receptacle about 140 μm long. Descending segment of loop entering albumen gland (ag) where it forks to form ventral canal (vc) of pallial oviduct (pov) leading forward to vagina, and a short broad duct leading posteriorly to bursa copulatrix (bu). Bursa copulatrix large and saccate, lying against posterior end of albumen gland. Ventral canal spiral in cross-section (Fig. 14), and continuous with lumen of capsule gland (cg). Albumen gland and capsule gland intricately coalesced to ventral canal, and together form the pallial oviduct. Pallial oviduct confined postero-laterally by posterior wall of mantle cavity (emc); irregularly pyriform in shape when viewed from above; bounded along its left side by intestine. Pallial oviduct terminating within mantle cavity just posterior to mantle collar and anus. Capsule gland and albumen gland not clearly differentiated superficially. Albumen gland occupying posterior third of pallial oviduct and consisting of tightly coalesced large glandular cells. Capsule gland consisting of smaller, and more compact cells. Ventral canal extending about a fourth of its length beyond anterior end of capsule gland.

Eggs laid singly in capsules on hard surfaces. Capsules hemispherical and attached to substrate by a narrow hyaline collar. Width of hemispherical capsule about 0.7 mm; width of collar about 0.05 mm; total width of capsule and collar about 0.8 mm.

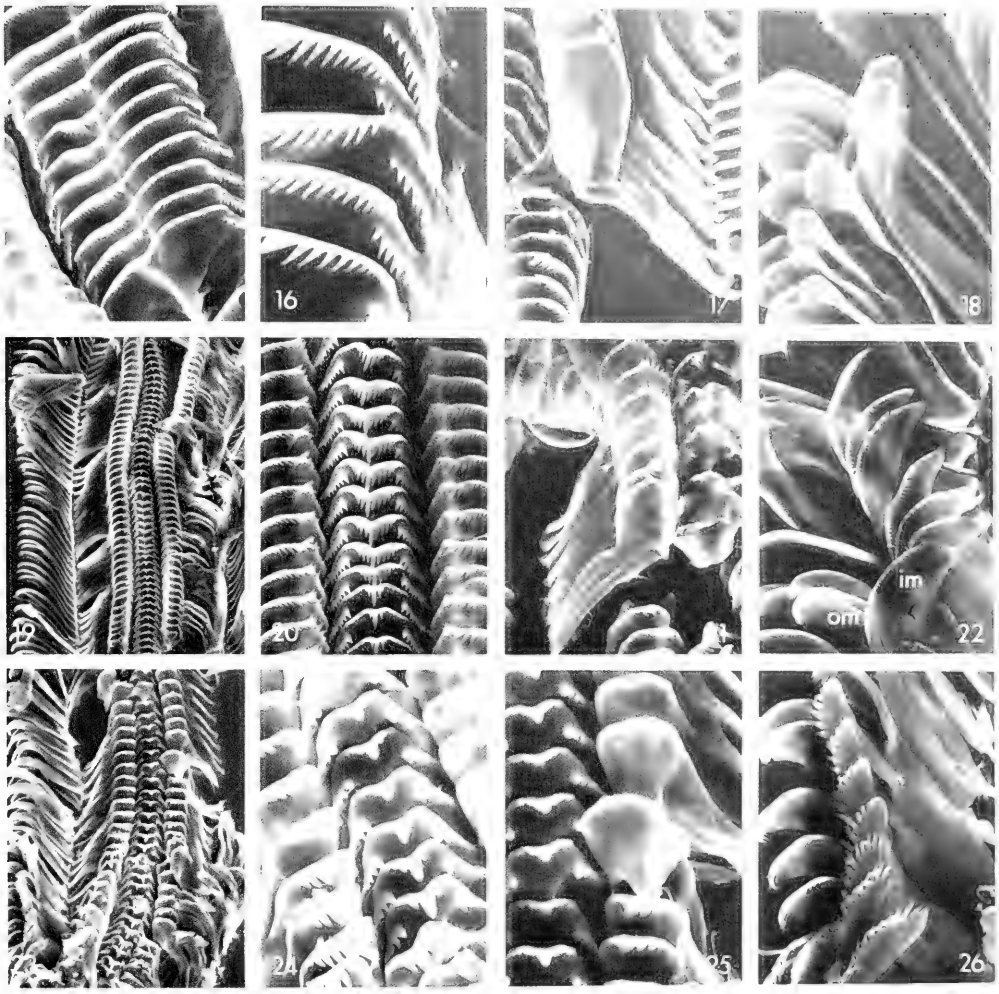
Male reproductive system (Figs. 2, 9). Testis (got) very large and completely covering posterior 1.5 whorls; overlying entire digestive gland, stomach and posterior third of prostate; testis consisting of numerous lobes that subdivide into small lobules that form a marbled pattern on outer surface. Lobes discharging into the primary sperm duct which forms a highly convoluted seminal vesicle (sv) on ventral surface of testis; this continues into posterior vas deferens (vd_1). The latter bearing 3–4 sigmoid coils near its middle. Posterior vas deferens passing mesad across ventral surface of body beneath junction of stomach and prostate, and then upward along right margin of pericardium, oesophagus and style sac to enter ventral side of prostate (pr) near its middle. Prostate with a relatively deep intestinal groove along its mesad curvature. Prostate completely posterior to transverse wall of mantle cavity (emc), but partially overlapping cavity. Anterior vas deferens (vd_2) leaving ventrocolumellar side of anterior edge of prostate, passing vertically down side of mantle cavity, and following an irregular sigmoid course across side of nape to base of penis (pe). Penis originating on right side of nape beneath mantle collar; long, flattened, blade-like, biconvex in cross section (Fig. 10) and unpigmented. When contracted, the penis is folded posteriorly and to the left within the mantle cavity. It bears a small patch of minute glands along its distal left margin on its ventral surface.

Clappia Walker, 1909

The genus was founded on a single species and was distinguished from *Somatogyrus* because of its open umbilicus (Fig. 68), its large opercular nucleus, and some characteristics of the radula. The sculpture of the protoconch remains unknown. The type-species, *C. clappi* Walker, 1909 (=*C. umbilicata* [Walker], 1904) from the Coosa River in Alabama apparently is extinct. A second species, *C. cahabensis* Clench, 1965, from the Cahaba River may also be extinct due to pollution from coal strip-mining in the area. Both species lived on high energy shoals.

External morphology and color. I have examined the dried bodies of 24 paratypes of *C. clappi* (ANSP 95037). The mantle is uniform black, similar to *Lepyrium* and most *Somatogyrus*. Walker (1909: 90) states that the animal is black. Presumably this refers to the foot, snout and nape, as well as the mantle. Dried males have a flattened, blade-like, unpigmented penis. Radulae were extracted from two specimens.

Radula. There are about 56–57 transverse tooth rows (Fig. 19). All teeth are characterized by having more numerous and smaller cusps than do those of *Somatogyrus*, but not to the extent that occurs in *Lepyrium*. Central tooth (Fig. 20) with moderately extended lateral angles; mesocone small, slender, acuminate, bordered on each side by 6–7 ectocones on a low ridge near outer edge of lateral angles; basocones subequal, gradually increasing in size dorsally. Lateral tooth (Fig. 21) with a strongly flexed, slender shaft; mesocone reduced in size, slender, bordered by 7–9 small entocones and 11–12 slender, subequal ectocones; basal projection long

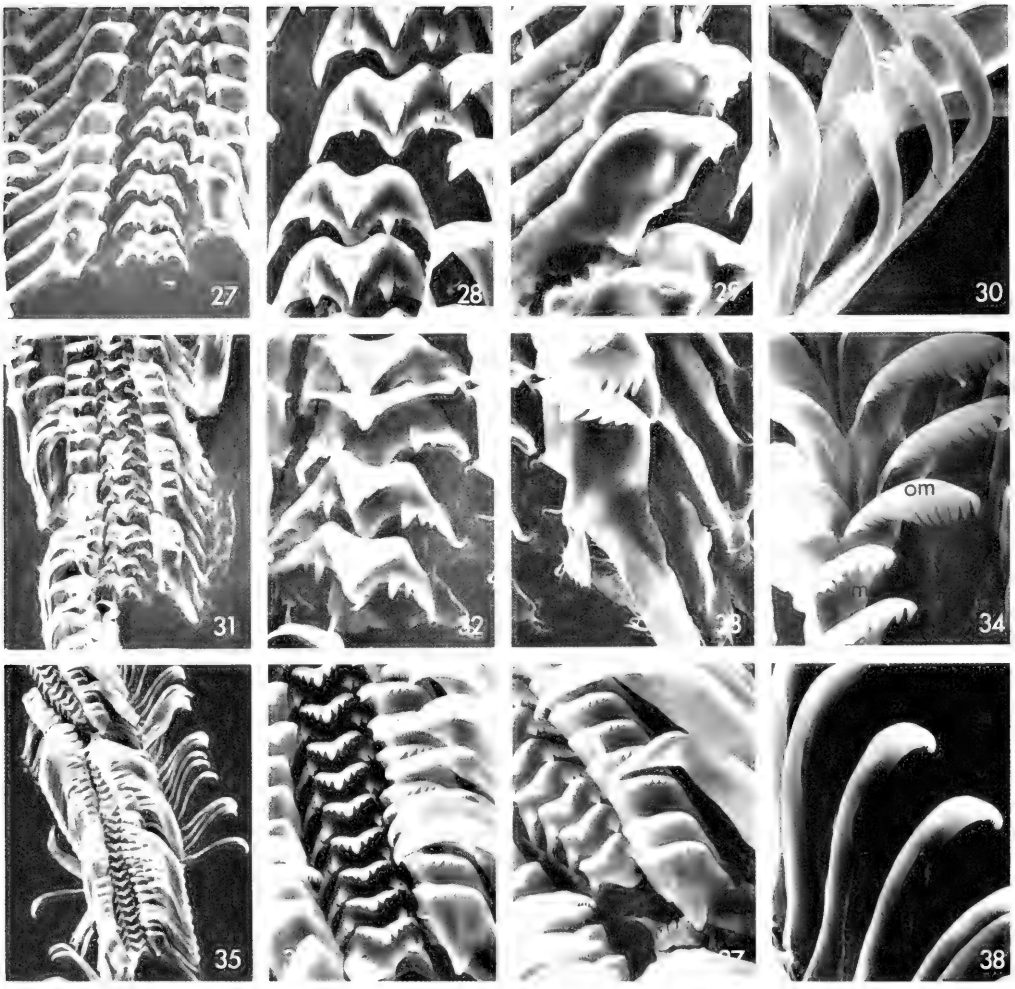


FIGS. 15–26. SEM photographs of radulae. Figs. 15–18. *Lepyrium showalteri* (Lea). Figs. 19–22. *Clappia umbilicata* (Walker). Figs. 23–26. *Somatogyrus depressus* (Tryon). Enlargements: Figs. 15, 17, 20, 21, 24, 25, 26 ×356; Figs. 19, 23 ×95; Figs. 18, 22 ×475; Fig. 16 ×950. Legends: im = inner marginal tooth; om = outer marginal tooth.

and slender. Inner marginal tooth (Fig. 22, im) with about 50 very small slender cusps. Outer marginal tooth (Fig. 22, om) with about 35 small slender cusps. Walker (1909: 89) stated that there are about 50, but the cusps are so small his count may have been only a rough approximation.

Somatogyrus Gill, 1863

The genus contains numerous species in eastern North America (Burch & Tottenham, 1980: 104–110). They have in common conical or ovate-conical shells 3–6 mm high. The protoconch has numerous fine spiral threads. Punctate sculpture may occur in addition (Fig. 40). Walker (1915) stated that some species have pitted sculpture only. I have not been able to confirm this among the material I examined. The columella generally is thickened. The thickness and structure of the columella, and the nature of the umbilicus provide useful characteristics for grouping species. The soft anatomy of the type-species, *S. depressus* Tryon, remains undescribed. The only preserved specimens that I examined were infected with trematode sporocysts,



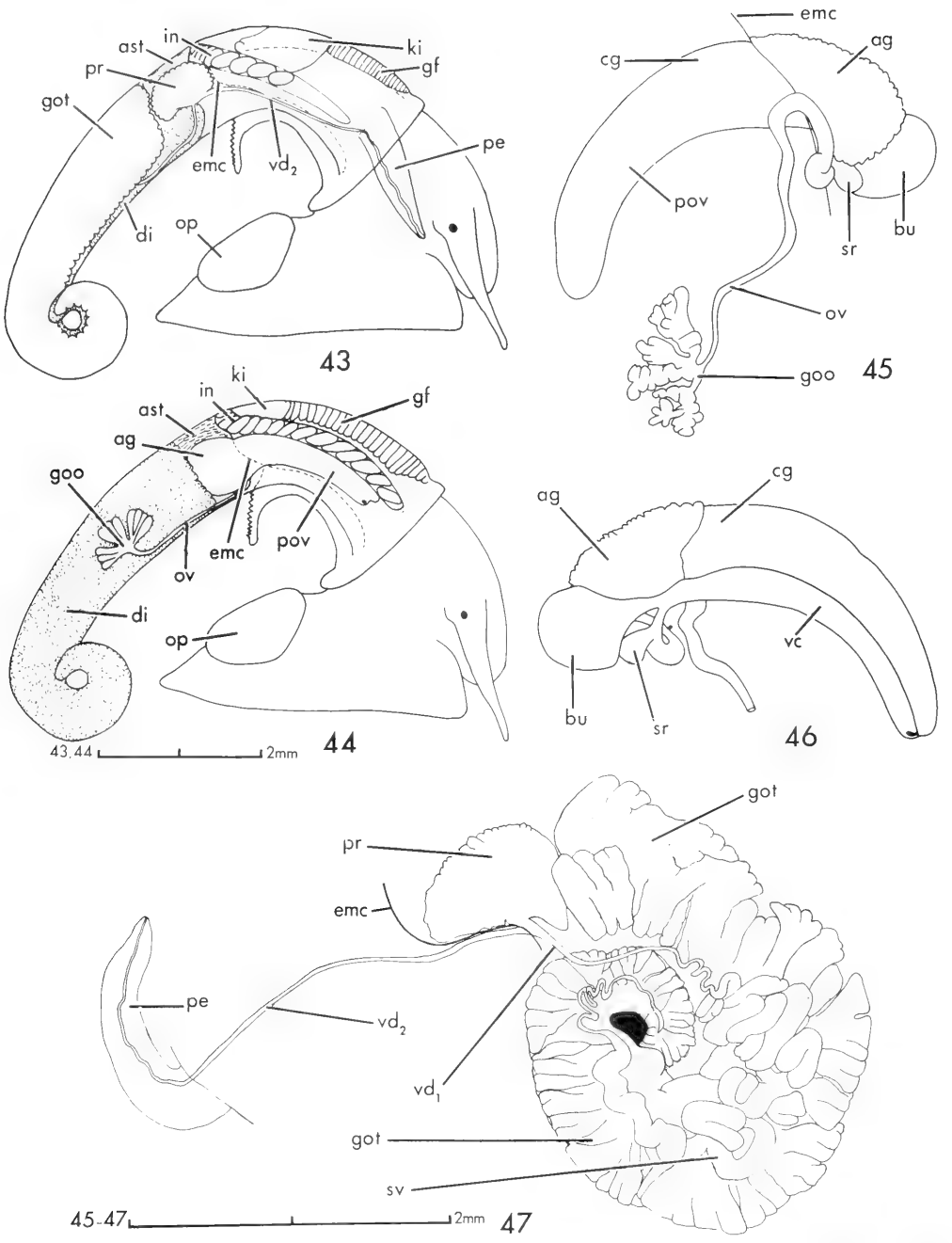
FIGS. 27–38. SEM photographs of radulae. Figs. 27–30. *Gillia altilis* (Lea). Figs. 31–34. *Fluminicola nuttalliana* (Lea). Figs. 35–38. *Somatogyrus rheophilus* n. sp. Enlargements: Fig. 27 ×143; Figs. 31, 35 ×95; Figs. 28–30, 32–34; 36–37 ×356; Fig. 38 ×475.

which grossly distorted the reproductive system. The radula of *S. depressus* was described by Stimpson (1865b: 21–22) who erred in reporting a perforation as occurring on the face of the lateral tooth. Walker (1909) used that as a characteristic to separate *Somatogyrus* from *Clappia*. Baker (1928: 148–149) correctly described and illustrated the radula of *S. depressus.* Its shell is illustrated in this paper (Figs. 65, 66) and its distribution is mapped (Fig. 71).

I have examined the anatomy of several species of *Somatogyrus* from Georgia, Alabama and Florida, and they are virtually identical in most features. I assume that *S. depressus* is not significantly different. Because of similarities in the radula between *S. depressus* (Figs. 23–26) and other species, e.g. *S. rheophilus* n. sp. (Figs. 35–38), aspects of the anatomy of *S. rheophilus* are described as representative for *Somatogyrus.* Some data for other species also are provided. The shell of *S. rheophilus* is described in Appendix A.

External morphology. Body, top of snout, sides of foot and dorsal surface of tentacles grayish black. Slight grayish patch present on each side of posterior base of tentacles. Underside of tentacles, muzzle, and sole grayish white. Mantle collar golden-flecked. Penis unpigmented. Outer surface of mantle cavity

FIGS. 39–42. SEM photographs of protoconchs, showing embryonic sculpture. Fig. 39. *Lepyrium showalteri* (Lea), ×77. Fig. 40. *Somatogyrus rheophilus* n. sp., ×116. Fig. 41. *Gillia altilis* (Lea), ×116. Fig. 42. *Fluminicola nuttalliana* (Lea), ×77.



FIGS. 43–47. *Somatogyrus rheophilus* n. sp. Fig. 43. Male denuded of shell and partially uncoiled. Fig. 44. Female denuded of shell and partially uncoiled. Figs. 45–46. Female reproductive system. Figs. 47. Male reproductive system. Legend: ag = albumen gland; ast = anterior chamber of stomach; bu = bursa copulatrix; cg = capsule gland; di = digestive gland; emc = posterior wall of mantle cavity; gf = gill filaments; goo = ovary; got = testis; in = intestine; ki = kidney; op – operculum; ov = oviduct; pe = penis; pov = pallial oviduct; pr = prostate; sr = seminal receptacle; sv = seminal vesicle; vc = ventral channel; vd_1 = proximal vas deferens; vd_2 = distal vas deferens.

with a large dark gray patch that is bounded along right side of pallial oviduct and posteriorly by hypobranchial gland. Patch variable from light gray to nearly black. Most species of *Somatogyrus* that I examined are similarly colored; a few species (undescribed) have black blotches and spots on the mantle. Gill lamellae about 26–34. Osphradium elongate, nearly as long as gill. Operculum ovate, paucispiral, with a subcentral nucleus.

Radula. Data for *S. rheophilus* are taken from SEM photos comprising Figs. 35–38 and from prepared slides. Data for *S. depressus* are presented in Table 1. Radula with about 35–45 transverse rows of teeth. Central tooth trapezoidal in shape with a mid-basal projection; mesocone enlarged, blunt, bordered by 3–4 blunt ectocones on each side; 3–4 basocones in a low ridge in middle of lateral angles; basocones increasing in size dorsally. Lateral teeth (Figs. 35–37 with 4 entocones, an enlarged mesocone and 5–6 ectocones; shaft elongate, slender, and flexed, though not as much as in *Lepyrium* and *Clappia*; basal projection stout, pointed. Inner marginal teeth (Fig. 37) stocky, with about 30 fine, acuminate cusps. Outer marginal teeth more slender, with about 30 fine cusps (Fig. 38).

Female reproductive system (Figs. 44–46). Ovary (goo) confined to upper whorl of viscera along columellar wall and completely imbedded in digestive gland (di) (Fig. 44); consisting of 3–4 lobes, each of which is subdivided into several smaller lobules. Primary oviduct (ov) passing along ventral-mesad side of digestive gland almost to posterior wall of mantle cavity (emc), and then passing posteriorly *to form an open loop along mesad side of albumen gland* (ag) (Fig. 45). Gono-pericardial duct short but stout. *Seminal receptacle* (Figs. 45–46, sr) *small, saccate, located on descending arm of oviduct loop; appressed against ventral side of bursa copulatrix (bu), but visible externally. Bursa copulatrix large, saccate, overlapping posterior end of albumen gland.* Duct from albumen gland joining primary oviduct at posterior partition of mantle cavity to form ventral canal (vc) of pallial oviduct (pov). *Capsule gland (cg) extending to end of ventral canal. Pallial oviduct usually more slender than in other genera.* Eggs deposited in single capsules on hard substrate. Egg capsule low, dome-shaped, 1.20–1.25 mm wide with a flat collar 0.20–0.25 mm wide.

Male reproductive system (Fig. 47). Similar in most aspects to *Lepyrium* except that *the penis lacks small dermal glands and is relatively more slender and blade-like.* Testis (got) very large, occupying upper two whorls of viscera, where it overlies dorsal surface of digestive gland, stomach, and posterior edge of prostate (pr, Fig. 43). Testes consisting of many large lobes that fork into 2–4 lobules each. Primary sperm duct lying along mid-ventral side of testis (Fig. 47). Its apical end very slender; in second visceral whorl primary sperm duct becomes greatly enlarged and convoluted forming a seminal vesicle (sv), and then narrowing to a thin delicate vas deferens (vd_1) above prostate. Prostate (pr) ovate in shape, imbedded in body wall just behind posterior wall of mantle cavity. Vas deferens (vd_2) imbedded in body wall for first half of its length, and then enters body cavity to base of penis (pe), where it courses through left side of penis and discharges at its tip. Penis originating on right side of nape behind right eye tentacle, sickle-shaped, dorso-ventrally flattened and recurved counterclockwise into the mantle cavity when contracted.

TABLE 1. Radular characteristics of some North American lithoglyphines and *Birgella*.

	Tooth rows	Central basocones	Central ectocones	Lateral cusps	Inner marginal cusps	Outer marginal cusps
Gillia altilis (7)	51–55	2	3–4	8–9	ca. 30	6–9
Fluminicola nuttalliana (5)*	—	2–3	4–5	7–8	ca. 16	12-13
Somatogyrus depressus (6)*	—	4	3–4	8–10	ca. 30	ca. 25
Somatogyrus rheophilus (7)	35–45	3–4	3–4	10–11	ca. 30	ca. 30
Clappia clappi (2)	56–59	6–7	6–7	18–21	ca. 50	ca. 35
Lepyrium showalteri (7)	140–149	9–11	22–24	18–22	ca. 50	ca. 50
Birgella subglobosa (2)	48–49	2	3–4	10–11	12	9

*SEM preparations; counts not taken.

Gillia Stimpson, 1865

Gillia is a monotypic genus found in streams along the Atlantic coast of eastern North America. Its shell is described and its distribution is discussed in Appendix A. The shell is characterized by its large size, ovate-conical shape, and fine spiral threads on the protoconch (Fig. 41).

External morphology. Similar to *Somatogyrus.* Mantle uniformly black or pigmentation may be reduced to a large fuscous blotch covering the mantle cavity and the stomach. Nape, top of snout, and top of tentacles black. Under side of tentacles white. Sides of foot and snout light gray. Operculum paucispiral, broadly ovate with a subcentral nucleus (Fig. 52). Baker (1918) described the eggs as laid singly or in small clusters at up to six on leaves and stems of aquatic plants. The capsules are hemispherical and 1.25 mm in diameter. An attachment collar is not mentioned or illustrated.

Radula (Figs. 27–30). The radula is specialized for feeding on coarser food particles than do the preceding genera. This is indicated by modifications of the central tooth (Figs. 27–28) and the lateral teeth (Fig. 29). The cusps on each tooth are modified and aligned to form a single large serrate blade. There is a correspoding loss of small cusps on the outer marginal tooth (Fig. 30) and among the basocones of the central tooth.

Central tooth (Figs. 27–28) broad and with greatly extended lateral angles; mesocone large, acuminate, bordered on each side by 3–4 ectocones that serve as serrations on the sides of the mesocone; basocones two on each side, the uppermost greatly enlarged and acuminate. Lateral tooth (Fig. 29) stout; shaft only slightly flexed, nearly aligned vertically with face of tooth; mesocone large, acuminate, bordered by 2 entocones and 4–5 sharp ectocones; basal projection stout, pointed. Inner marginal tooth with about 30 cusps. Outer marginal tooth (Fig. 30) slender, with about 6 relatively stout cusps.

Female reproductive system. The female system (Figs. 48–50) is similar to that of *Somatogyrus* and *Fluminicola*, and differs from *Lepyrium* in the size and location of the seminal receptacle (sr) and the extent of the capsule gland (cg). The loop of the oviduct may or may not be folded along the side of the albumen gland (ag). The seminal receptacle originates at the base of the oviduct loop and lies along the lower edge of the bursa copulatrix (bu), and like *Fluminicola* it is completely covered by albumen gland (ag) tissue. The capsule gland (cg) extends to the end of the ventral canal.

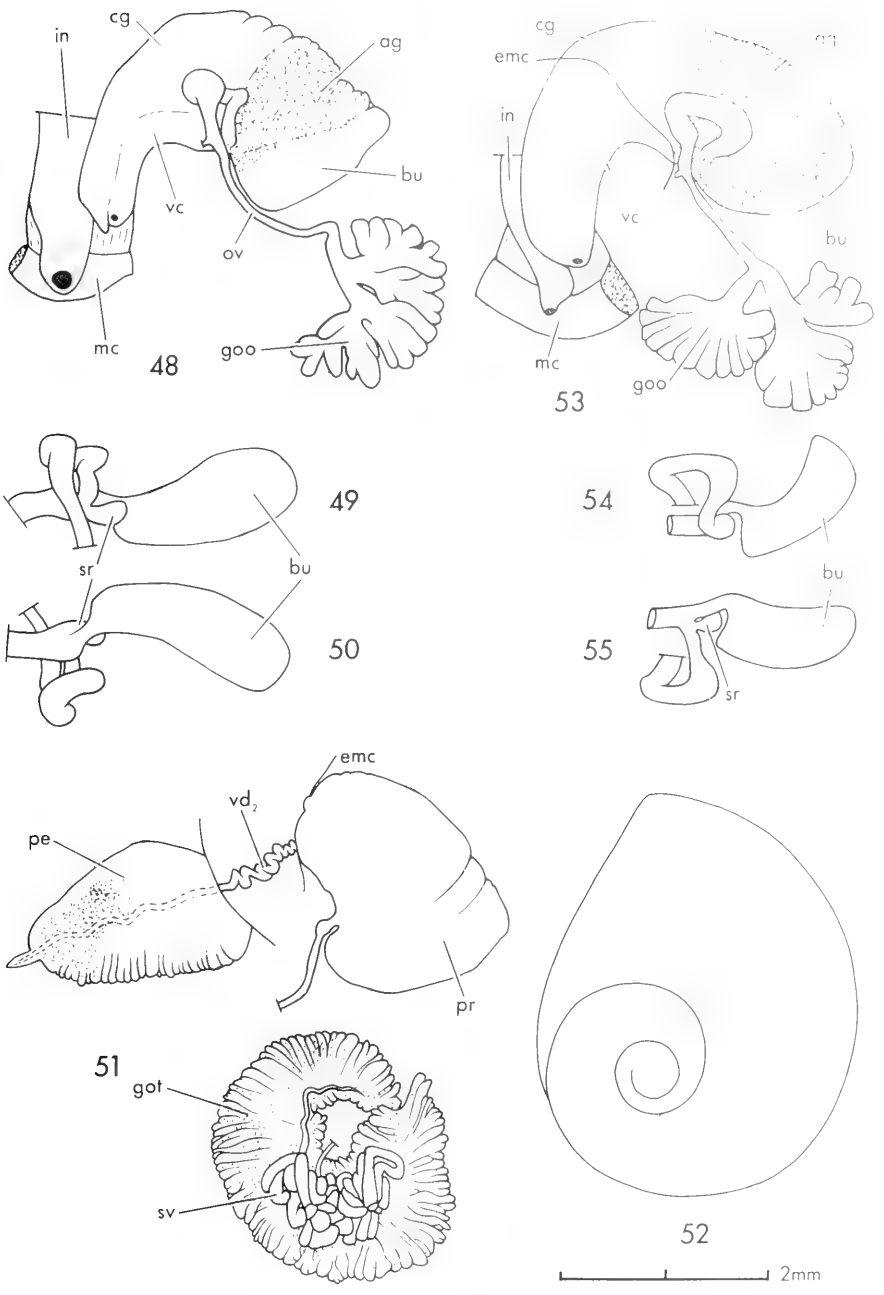
Male reproductive system. Gillia (Fig. 51) differs from *Lepyrium* and *Somatogyrus*, but is like *Fluminicola* in the extent of the testes and the pigmentation and shape of the penis (pe). The testis (got) extends forward only to the posterior half of the stomach and to the posterior edge of prostate (pr). Anterior vas deferens (vd_2) highly convoluted, passing along right side of body wall and then transversely across nape to base of penis. Penis flattened, blade-like (contracted in Fig. 51), and terminating in a slender fleshy papilla through which the vas deferens discharges; distal third of penis with an internal patch of melanophores along vas deferens.

Fluminicola Stimpson, 1865

The specimens I examined are tentatively identified as *F. nuttalliana* (Lea), the type-species of *Fluminicola.* They are typical in all aspects except that they are smaller than average. I have not seen sufficient material to review the systematics of *F. nuttalliana* nor to discuss its distribution. The shells of *Fluminicola* vary greatly in size, up to 12 mm. The shell varies from ovate-conical to globose. The protoconch of *F. nuttalliana* (Fig. 42) is similar to *Gillia* by having fine spiral sculpture.

External morphology. Animal like *Gillia.* Mantle uniformly black. Operculum like *Gillia.* Eggs laid on hard objects, single or in small clusters; hemispherical, 1.25 mm in diameter, with a narrow hyaline collar 0.12–0.15 mm wide.

Radula. The radula (Figs. 31–34) is similar to that of *Gillia* by having greatly enlarged cusps on the central and lateral teeth. It differs from *Gillia* primarily by the number of cusps on the marginal teeth. Central tooth with extended lateral angles; upper edge (Fig. 32) with an enlarged acuminate mesocone bordered on each side with 4–5 ectocones that combine to form a large, serrated, projecting blade; uppermost basocone greatly enlarged, followed by 1–2 much smaller basocones on each side. Lateral tooth (Fig. 33) with a weakly flexed, stout shaft, and an enlarged mesocone bordered by two smaller entocones and four ectocones; basal projection long, slender, stout. Inner marginal tooth (Fig. 34) with about 16 acuminate cusps.



FIGS. 48–55. *Gillia altilis* (Lea). Fig. 48. Female reproductive system. Fig. 49. Coil of oviduct freed from albumen gland showing seminal receptacle. Fig. 50. Right side of oviduct coil showing relationship of bursa copulatrix to seminal receptacle. Fig. 51. Male reproductive system. Fig. 52. Operculum. Figs. 53–55. *Fluminicola nuttalliana* (Lea). Fig. 53. Female reproductive system. Fig. 54. Coil of oviduct freed from albumen gland. Fig. 55. Right side of oviduct coil showing relationships of seminal receptacle and bursa copulatrix. Legend: ag = albumen gland; bu = bursa copulatrix; cg = capsule gland; emc = posterior wall of mantle cavity; goo = ovary; got = testis; in = intestine; mc = mantle collar; ov = oviduct; pe = penis; pr = prostate; sr = seminal receptacle; sv = seminal vesicle; vc = ventral channel; vd_2 = vas deferens.

Outer marginal tooth (Fig. 34, om) with about 12–13 relatively stout cusps.

Female reproductive system. Similar to that of *Gillia altilis* except that the loop of the primary oviduct is not secondarily folded (Figs. 53–55). In contrast to other genera, the pallial oviduct lies lower along the right side and the intestine occupies a dorsal position over the pallial oviduct.

Male reproductive system (not figured). Most similar to *Gillia altilis*. The testis extends forward to overlap the posterior half of the stomach and the posterior margin of the prostate. Penis pigmented internally with two diffuse bands of melanophores along the distal third of the vas deferens, and tip of penis ending in a small fleshy papilla.

My observations on the penis differ from the description given by Stimpson (1865b: 24–26). He stated that the left base of the penis has a wing-like expansion. However, Stimpson's material came from an unspecified locality and was poorly preserved. Thus the identity of the species he examined is uncertain.

PHYLOGENETIC RELATIONSHIPS

The genera discussed above have been classified in two orders and three families within the Subclass PROSOBRANCHIA. *Lepyrium* was first considered to be in the Neritidae, a family in the Order ARCHAEOGASTROPODA, because of its neritid-shaped shell (Lea, 1861; Binney, 1865; Dall, 1896; Walker, 1918; Wenz, 1939). Pilsbry & Olsson (1951) established its affinities to the order MESOGASTROPODA on the basis of its taenioglossate radula. They proposed the monotypic Family Lepyriidae because the combination of its radular and opercular characters was dissimilar to other families of freshwater gastropods. The genera *Somatogyrus, Clappia, Gillia,* and *Fluminicola* traditionally have been grouped together in the mesogastropod family HYDROBIIDAE, Subfamily LITHOGLYPHINAE because of their thick, globose, lithoglyphine-type shells (Stimpson, 1865b; Walker, 1918; Burch & Tottenham, 1980). This relationship is correct but for the wrong reason.

The globose lithoglyphine-type shell is an adaptation for two very different habitats, and has evolved at least four times in unrelated hydrobioid subfamilies. It has evolved independently in the Pomatiopsidae (Triculinae) and the Hydrobiidae (Lithoglyphinae) as an adaptation for existence in high-energy streams. The globose shell accommodates an enlarged foot and muscle system for attachment to rocks in swift currents (Davis, 1979; Davis & da Silva, 1983; this paper). A similar type of shell has evolved twice again within the hydrobiid subfamily Nymphophilinae as an adaptation to a very different habitat. *Birgella* and *Notogillia*, two distantly related genera within the subfamily, live in quiet waters on fine-particle substrates (Thompson, 1968; this paper). A wide foot is required to support the snail's weight on a silt substrate, and the enlargement of the foot is accommodated by an enlarged globose shell. It is clear that the lithoglyphine-type shell is highly adaptive, and thus is convergent. Conclusions concerning suprageneric relationships based on this character-state must take convergence into account. During recent years, as knowledge about the anatomy of hydrobioid snails progressed, it has become increasingly difficult to define family units (families and subfamilies) (Taylor, 1966; Davis, 1966; Thompson, 1968; Radoman, 1973; Davis, 1979; Thompson, 1979; Hershler & Davis, 1980; Davis, *et al.,* 1982; Davis & Pons da Silva, 1983). With increasing knowledge about the anatomy of additional genera the distinctions between family units becomes less clear, and requires redefinition of established units or the designation of new units. The result is that family units are becoming separable by fewer and fewer characteristics, and frequently their definitions include words such as "except," "as in," and "shared with." Such instability is expected because fewer than 20% of the hydrobioid genera have been studied to the extent that the internal morphology is known for a single species. It is clear that the classification of the hydrobioids is in an embryonic state of knowledge. A great deal more must be learned before any stability in classification can be achieved. Such a classification will have to be based on consistent criteria of morphology, biochemistry, genetics, behavior, and ecology. At present the hydrobioids are classified only on morphological criteria that are proving not to be consistent. None-the-less the employment of such criteria is useful in deriving phylogenetic concepts.

Family relationships. On the basis of anatomical data it is clear that *Lepyrium, Clappia, Somatogyrus, Gillia* and *Fluminicola* constitute a compact monophyletic group within the Hydrobiidae as defined by Davis (1979).

The family relationship is established by seven morphological characters. The taenioglossate radula has a trapezoidal central tooth with pronounced lateral angles extending beyond the posterior margin. The central tooth has two or more pairs of ectocones on each side of the mesocone. Epitaenia and associated food grooves for filter feeding are absent. Sperm enters the female reproductive system through the genital pore at the anterior end of the pallial oviduct where it passes posteriorly *via* a ciliated ventral canal (except Littoridininae; Hershler, in press). The single seminal receptacle originates on the primary oviduct posterior to the bursa copulatrix. The eggs are deposited singly in tough hemispherical capsules and are not coated with sand. The prostate overlaps the posterior edge of the mantle cavity. Two other characters, though not unique to the Hydrobiidae, also serve to remove the group from other superfamilies. The simple, chitinous, paucispiral operculum lacks an internal peg. Sperm transmission from the male is accomplished through a penis that originates on the nape and is innervated by the pleuropedal connective.

Subfamily relationships. Within the Hydrobiidae six subfamilies presently are recognized in North America. Lithoglyphinae (this paper; Davis and Pons da Silva, 1983), Nymphophilinae (Thompson, 1979; Hershler, in press), Littoridininae (Davis *et al.,* 1982; Hershler, in press), Hydrobiinae (Davis, 1966; Hershler & Davis, 1980), Amnicolinae (Thompson, 1968), and Fontigentinae (Burch, 1982). The last two subfamilies are excluded from further discussion because of the presence of two (Amnicolinae) or three (Fontigentinae) ducts within the penis. They are considered remote in their relationships to the other subfamilies though their anatomies are poorly known. Eight character-states common to the *Lepyrium*-group of genera are useful for establishing relationships within the remaining subfamilies and for defining the subfamily Lithoglyphinae. (1) The shell is globose or conico-globose. (2) The protoconch is sculptured with spiral lirations; spirally arranged series of pits may occur in addition. (3) The mantle is uniform black or dark gray. (4) One or more pairs of basal cusps arise from the face of the radular central tooth, not the lateral angles. (5) The stomach lacks folds or protuberances on its posterior chamber. (6) The fecal pellets are cylindrical and are spiral in structure. (7) The penis is flattened and blade-like, with a simple duct (vas deferens) internally. (8) The penis lacks lobes or complex glandular structures on the outer surface.

Within the Hydrobiidae characters 4, 5, 6, and 7 are exclusive to the Lithoglyphinae. Character 3 may also be. I am not aware of data to the contrary. The remaining character-states are shared with one or more subfamilies.

The globose or conico-globose lithoglyphine-type shell also occurs in some Nymphophilinae and Littoridininae. In those subfamilies, it is an uncommon character. All of the genera that have been shown to be Lithoglyphinae because of their soft anatomies have lithoglyphine-type shells. Two conclusions are suggested by this. The lithoglyphine-type shell in the Lithoglyphinae is fundamental, and thus is a primitive character-state. Also, the habitat deployment of the Lithoglyphinae in high-energy streams is a basic, and therefore primitive behavioral characteristic of the subfamily. Some species, *e.g. Somatogyrus depressus* (Tryon) and *Gillia altilis* (Lea) may inhabit quiet-water habitats as well as high-energy streams. This can be considered a secondary adaptation because it occurs seldom and sporadically with the subfamily, not among a cluster of closely related species, and it occurs among species that also inhabit fast streams.

The spiral sculpture of the protoconch is characteristic of the Lithoglyphinae. A secondary reduction may occur (*Lepyrium*) or secondary additions may occur (*Somatogyrus*). Spiral protoconch sculpture in *Birgella* (Figs. 79–80) is the only recorded occurrence in the Nymphophilinae. Spiral protoconch sculpture also occurs in some southeastern Amnicolinae, *e.g. Lyogyrus retromargo* (Thompson). This type of sculpture may be a primitive condition within the Hydrobiidae, with other types of sculpture representing derived states.

The absence of glandular ridges, raised glands, lobes or papillae on the penis is a character state that also occurs in some Littoridininae (Hershler, in press) and in some Hydrobiinae (Hershler & Davis, 1980). In those cases it may be a derived condition through the secondary loss of previously existing characters. The total absence of these structures in the Lithoglyphinae suggests that their absence is a generalized, primitive condition in the subfamily.

The Lithoglyphinae as presently understood includes seven genera. Five in

North America, *Potamolithus* in South America (Davis & Pons da Silva, 1983) and *Lithoglyphus* in Europe (Krause, 1949). In addition to the nine character-states listed above that differentiate North American lithoglyphines from other Hydrobiidae, the North American genera have in common five character-states that differentiate them from other Lithoglyphinae. In sequence with the character-states listed above these are as follows: (9) The testis is very large; it covers almost the entire dorsal surface of the digestive gland and partially overlaps the stomach and prostate. (10) The vas deferens is not modified into an enlarged ejaculatory duct at the base of the penis. (11) The penis may or may not have a terminal papilla; when present the papilla is non-retractable. (12) The penis lacks a preputium. (13) A nuchal node is absent.

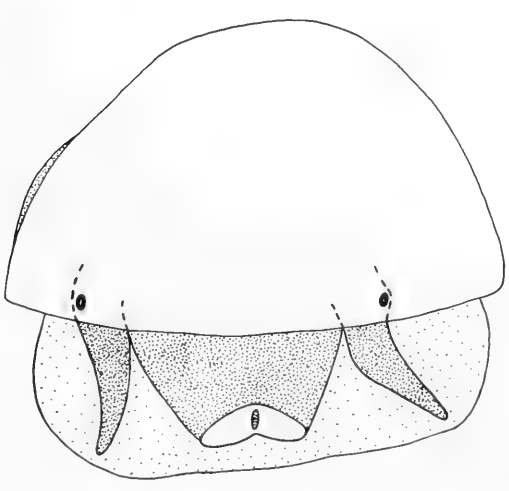
The size of the testis separates the New World genera from *Lithoglyphus*. In *Lithoglyphus,* Krause (1949) describes the testis as overlying the digestive gland in the first and second whorl. The extent of its distribution in these two whorls is not clear. His illustration (p. 135, fig. 22) depicts a testis that is not larger than the prostate. If that is correct, the testis is very small compared to those in other genera. The remaining four character-states differentiate the North American genera from *Potamolithus* (see Davis & Pons da Silva, 1983).

THE NORTH AMERICAN LITHOGLYPHINE GENERA

The evolution of North American Lithoglyphinae centered about microhabitat selection and trophic specialization. Microhabitat selection produced variation in shell-form within the constraints imposed by a lentic environment. Trophic specialization is reflected in variations of the basic structure of the cusps on the radular teeth and the shaft of the lateral tooth. Character-trends are discernible in shell form and radular structures.

Shell form. A high degree of diversity exists in the shells of North American Lithoglyphinae. The species vary in size from small to large (2–12 mm) and may be obese with a short or depressed spire, ovate-conical with a pronounced spire, or flattened and limpet-like. They include the largest of the American Hydrobioidea. Most have a voluminous body whorl. Basically, most shells are imperforate or narrowly rimate. When viewed from the front, most have a noticeable spire protruding from the right side (Fig. 57). When the animal is active, the shell is raised and the eyes, tentacles, and muzzle extend considerably beyond the edge of the lip.

Two lines of specialization in shell form occur. *Clappia* diverges from the basic shell form by having a broadly perforate umbilicus (Fig. 68). The adaptive significance of this is



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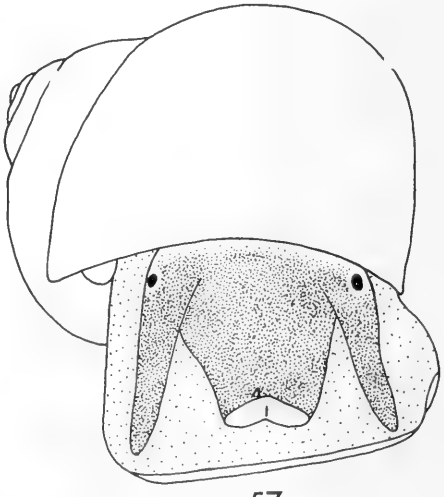

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FIG. 56–57. Fig. 56. Anterior view of *Lepyrium showalteri* (Lea); note reduction of spire to produce a limpet-like shell. Fig. 57. Anterior view of *Somatogyrus rheophilus* n. sp., a lithoglyphine with a normal-spired shell.

not clear because little information is available on the ecology of *Clappia*. However, this is only a variation in degree from the perforate umbilicus of some *Somatogyrus* (Fig. 65). *Lepyrium* diverges from the basic shell form by being limpet-like, with a depressed spire and a greatly enlarged aperture. When the animal is viewed from the front, the shell covers the body like a low shield and the spire is barely evident (Fig. 56). The animal raises its shell only slightly as it moves, and the eyes, tentacles and snout barely protrude beyond the edge of the lip. The shell form of *Lepyrium* is a modification for reducing hydrostatic drag on an animal that lives in fast currents on smooth boulders, where characteristically it is found. The neomelanian type operculum of *Lepyrium* is a secondary specialization related to the enlarged aperture.

Other variations in shell morphology are significant as species-level criteria or species-group criteria. The large size of *Gillia altilis* readily distinguishes this monotypic genus from other eastern North American genera, but not from *Fluminicola,* which achieves an even larger size. However, it is not shell size by which *Gillia* is separated from other genera, but by trophic structures. Furthermore, some of the more globose species now placed in *Somatogyrus* may be found to belong in *Gillia* when their radulae are examined.

Radula. Three important character-trends occur in trophic structures. They are the primary modification through which adaptive radiation of the Lithoglyphinae has taken place in North America. Each genus is characterized more by its radular features than by other structures. These features indicate different feeding roles for the various genera. Radular data are summarized in Table 1. The trends are: (1) modifications in the number of transverse tooth rows, (2) modifications of size and numbers of cusps on the radular teeth, and (3) a corresponding modification in the size and orientation of the shaft of the lateral tooth.

Most species have a moderate number of transverse tooth rows, about 35–55 (Table 1, Fig. 23). In two groups, *Clappia* and *Lepyrium*, there is a significant increase in the number of rows. This increase is accommodated by a greater degree of overlap between successive rows, not by increasing the relative length of the ribbon (Figs. 15, 19).

Two trends can be recognized in modifications of size and numbers of radular cusps. In *Clappia* and *Lepyrium* there is a decrease in the relative size of the cusps accompanied by an increase in the number of cusps on each tooth, indicating that these genera are specialized for grazing on finer plant-food particles than does the related genus *Somatogyrus*. A second trend occurs in *Gillia* and *Fluminicola*. In these genera the basocones in the central tooth are decreased in number, but are greatly enlarged, as are other cusps on the central and lateral teeth. There is also a corresponding decrease in the number, but an increase in relative size of the cusps on the inner and outer marginal teeth. These are specializations for grazing on coarser food materials than do related genera.

The shaft of the lateral tooth is moderately stout and is flexed laterally at a slight angle to the tooth face in *Somatogyrus* (Figs. 23, 25). In genera with reduced cusp size (*Clappia* and *Lepyrium*) the shaft is slender and is flexed laterally at a greater angle (Figs. 21, 17). This accommodates an increase in transverse tooth rows without increasing the relative length of the ribbon. In genera with enlarged cusps (*Gillia* and *Fluminicola*) the shaft is stouter and is aligned almost vertically with the face of the tooth (Figs. 29, 33). These changes are related to trophic specializations. Those genera that feed on coarse foods require stout shafts with rectilinear vertical support for the lateral tooth. Those genera that feed on smaller food particles require less support and can function with slender, strongly flexed lateral tooth shafts.

Relationships. Ten character-states are useful for determining intergeneric relationships. These states are discussed at various points earlier in this paper and are summarized in Table 2. A phenogram based on these character-states is depicted in Fig. 58. *Gillia* and *Fluminicola* have eight character-states in common (1–8). They differ from each other in two character states (9–10). They differ exclusively from the other genera by six character-states (4–9). *Somatogyrus, Clappia,* and *Lepyrium* have three character-states in common (4, 5, 10). *Somatogyrus* differs from the *Clappia-Lepyrium* lineage by three character-states (7, 8, 9). *Clappia* and *Lepyrium* have three character-states in common (7, 8, 9). They differ from each other in four character-states (1, 2, 3, 6).

From the data presented in Table 2 it is apparent that modification of feeding struc-

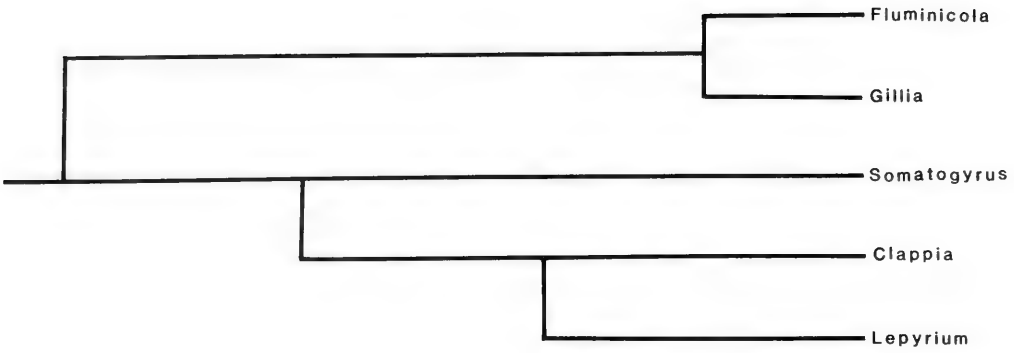

FIG. 58. Phenogram depicting intergeneric relationships of North American Lithoglyphinae based on characters listed in Table 2.

TABLE 2. Variation in ten character-states among North American lithoglyphine genera.

	Somatogyrus	*Clappia*	*Lepyrium*	*Gillia*	*Fluminicola*
Shell shape ovate-conical (0) neritid (1)	0	0	1	0	0
Umbilicus imperforate-rimate (0) open (1)	0	1	0	0	0
Operculum subcentral (0) excentric (1)	0	0	1	0	0
Size large (<5 mm) (0) small (> mm) (1)	1	1	1	0	0
Penis papilla present (0) absent (1)	1	1	1	0	0
Central tooth cusps large (0) medium (1) small (2)	1	1	2	0	0
Lateral cusps large (0) medium (1) small (2)	1	2	2	0	0
Lateral shaft vertical (0) angular (1) flexed (2)	1	2	2	0	0
Inner marginal cusps large (16) (0) medium (30) (1) minute (50) (2)	1	2	2	1	0
Outer marginal cusps large (6–9) (0) medium (12) (1) small (25+) (2)	2	2	2	0	1

tures (trophic specialization) is the fundamentally most important factor underlying the adaptive radiation of the Lithoglyphinae in North America. This is coupled with a minor degree of variation in the structure of the penis (reproductive specialization). It is also apparent that variation in the shell and operculum are significant at lower taxonomic levels, and that they are adaptations reflecting microhabitat specialization.

The North American lithoglyphine genera are redefined as follows. They have in common the characters discussed earlier in this paper, which differentiates them from European and South American genera.

Gillia Stimpson, 1865a

Type-species. Melania altilis Lea, 1841 (see Figs. 63, 64; Appendix A).

Definition. Shell medium to large (6–8 mm high). Imperforate or rimate. Conico-globose in shape. Protoconch sculptured with spiral striations. Operculum paucispiral. Penis with a small terminal papilla. Shaft of lateral tooth straight. Cusps of radular teeth enlarged; numbers of cusps given in Table 1. Outer marginal tooth with few (6–9) cusps.

Distribution. Atlantic drainage systems of eastern North America from South Carolina north to New York and Vermont.

Species. Monotypic.

Fluminicola Stimpson, 1965a

Type-species. Paludina nuttalliana Lea, 1839 (see Burch & Tottenham, 1980: 101, fig. 142, for an excellent illustration of the species.

Definition. Shell medium to large in size (up to 12 mm high). Imperforate or rimate. Conico-globose or globose in shape. Protoconch sculptured with spiral striations. Operculum paucispiral. Penis with a small terminal papilla. Shaft of lateral tooth straight. Cusps on radular teeth enlarged; numbers of cusps given in Table 1. Inner marginal tooth with few (16) cusps.

Distribution. Pacific drainage systems from California north to Washington and interior basin.

Species. Indeterminate. Burch & Tottenham (1980: 102) list 12 species. The radula and soft anatomy of only the type-species is known.

Note. Taylor (1966) synonymizes *Fluminicola* with *Lithoglyphus.* In light of the differences in the prostate gland and the radular cusps, they are retained as separate genera pending additional anatomical data on other species of *Fluminicola.* Radoman (1966) figures the radula of *L. naticoides* (C. Pfeiffer) as having few cusps on the inner marginal tooth (8–9) and on the outer marginal tooth (7).

Somatogyrus Gill, 1863

Type-species. Amnicola depressa Tryon, 1862 (see Figs. 65, 66).

Definition. Shell small to medium in size (1–6 mm high). Imperforate, rimate or narrowly umbilicate. Conico-globose or globose in shape. Protoconch sculptured with spiral threads, and it may also have spirally arranged series of pits. Operculum paucispiral with a sub-lateral nucleus. Penis without distinct terminal papilla. Shaft of lateral tooth weakly angular. Cusps of radular teeth moderately large; numbers of cusps given in Table 1.

Distribution. Eastern North America throughout the Mississippi drainage system, and from the Potomac River south and west through the Gulf Coast systems. Panuco River system of Mexico.

Species. Numerous. Burch & Tottenham (1980) list 35 species in the United States. Pilsbry (1910) describes a species from the Rio Coy in Mexico. Another is described in this paper.

Remarks. Species may differ by the number and size of the cusps on the radular teeth and the number of tooth rows. Two subgenera have been recognized on the basis of cusp development: *Somatogyrus s. s.* and *Walkerilla* Thiele, 1928. The degree of cusp development does not seem to be an adequate feature for separating subgenera. Convergence in this characteristic among species of quite dissimilar shells (*S. coosaensis* and *S. tenax*; see Burch & Tottenham, 1980) suggests that *Walkerilla*, as used previously (Thompson, 1969: 260), is polyphyletic and artificial in concept.

Clappia Walker, 1909

Type-species. Clappia clappi Walker, 1909 (= *Somatogyrus umbilicatus* Walker, 1904) (Fig. 68).

Definition. Shell small (about 3 mm high). Broadly umbilicate. Conico-globose in shape. Protoconch sculpture unknown. Operculum

paucispiral with a large subcentral nucleus. Penis simple, apparently without a terminal papilla. Shaft of lateral tooth strongly flexed. Cusps of lateral and marginal teeth minute; numbers of cusps given in Table 1.

Distribution. Confined to the Coosa and Cahaba rivers in central Alabama.

Species. Two; both may be extinct.

Lepyrium Dall, 1896

Type-species. Neritina showalteri Lea, 1861 (see Figs. 59–62; Appendix A).

Definition. Shell medium in size (about 4 mm high). Imperforate. Flattened, neritid in shape, but with a complete internal spire; aperture greatly enlarged for a limpet-like mode of existence. Protoconch smooth with a few low, wide, spiral grooves. Operculum paucispiral with a very excentric nucleus (neomelanian, Fig. 7). Penis simple, without a terminal papilla. Shaft of lateral tooth strongly flexed. Cusps of all radular teeth minute, accompanied by a large proliferation of transverse tooth rows. Numbers of cusps given in Table 1.

Distribution. Coosa, Cahaba, and Little Cahaba rivers in central Alabama.

Species. Monotypic.

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LITERATURE CITED

BAKER, F. C., 1902, The Mollusca of the Chicago area: the Gastropoda. *Bulletin of the Chicago Academy of Sciences,* 3: 137–410, pl. 28–35.

BAKER, F. C., 1918, Notes on nidification in *Gillia* and *Amnicola. Nautilus,* 32: 19–23, pl. 2, figs. 1–10.

BAKER, F. C., 1926, Nomenclatural notes on American freshwater Mollusca. *Transactions of the Wisconsin Academy of Arts, Sciences, and Letters,* 22: 193–205.

BAKER, F. C., 1928, The freshwater Mollusca of Wisconsin: Gastropoda. *Bulletin Wisconsin Geological and Natural History Survey,* 70: i–xvii, 1–507, 38 pl.

BERRY, E. G., 1943, The Amnicolidae of Michigan: distribution, ecology and taxonomy. *Miscellaneous Publications, Museum of Zoology, University of Michigan,* 57: 1–68, 9 pl.

BINNEY, W. C., 1865, Land and freshwater shells of North America: Ampullariidae, Valvatidae, Viviparidae, freshwater Rissoidae, Cyclophoridae, Truncatellidae, freshwater Neritidae, Helicinidae. *Smithsonian Miscellaneous Collections,* 144: i–viii, 1–120.

BURCH, J. B., 1982, Freshwater snails (Mollusca, Gastropoda) of North America. *Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency:* i–vi, 1–294.

BURCH, J. B. & TOTTENHAM, J. L., 1980, North American freshwater snails: species list, ranges and illustrations. *Walkeriana,* 3: 81–215.

CLARKE, A. H., 1981, *The freshwater Mollusca of Canada.* National Museum of Natural Sciences, National Museums of Canada: 1–446.

DALL, W. H., 1896, Notes on *Neritina showalteri. Nautilus,* 10: 13–15.

DAVIS, G. M., 1966, Notes on *Hydrobia totteni. Venus, Japanese Journal of Malacology,* 25: 27–42.

DAVIS, G. M., 1979, The origin and evolution of the gastropod family Pomatiopsidae, with emphasis on the Mekong River Triculinae. *Monograph of Academy of Natural Sciences, Philadelphia,* 20: 1–120.

DAVIS, G. M. & PONS DA SILVA, M. C., 1984, *Potamolithus:* morphology, convergence, and relationships among hydrobioid snails. *Malacologia,* 25: 73–108.

DAVIS, G. M., MAZURKIEWICZ, M. & MANDRACCHIA, M., 1982, *Spurwinkia:* morphology, systematics, and ecology of a new genus of North American marshland Hydrobiidae (Mollusca: Gastropoda). *Proceedings of the Academy of Natural Sciences of Philadelphia,* 134: 143–177.

GILL, T., 1863, Systematic arrangement of the mollusks of the family Viviparidae, and others, inhabiting the United States. *Proceedings of the*

Academy of Natural Sciences of Philadelphia, 15: 33–40.

GOODRICH, C., 1941, Distribution of the gastropods of the Cahaba River, Alabama. *Occasional Papers, Museum of Zoology, University of Michigan,* 428: 1–30.

HALDEMAN, S. S., 1847–1848, G. *Leptoxis.* Leptoxe. Rafinesque. *In*: CHENU, J. C., ed., *Illustrations Conchyliologiques.* Franck, Paris, 3: 1–6, 5 pl.

HERSHLER, R., in press, The systematics and evolution of the hydrobiid snails (Gastropoda: Rissoacea) of the Cuatro Cienegas basin, Coahuila, Mexico. *Malacologia,* 26: 000–000.

HERSHLER, R. & DAVIS, G. M., 1980, The morphology of *Hydrobia truncata* (Gastropoda: Hydrobiidae): relevance to systematics of *Hydrobia. Biological Bulletin,* 158: 195–219.

KRAUSE, H., 1949, Untersuchungen zur Anatomie and Okologie von *Lithoglyphus naticoides* (C. Pfeiffer). *Archiv für Molluskenkunde,* 78: 103–148.

LEA, I., 1839, Descriptions of new fresh water and land shells. *Transactions of the American Philosophical Society,* 6: 1–111, 24 pl.

LEA, I., 1841, Continuation of Mr. Lea's paper on new fresh water and land shells. *Proceedings of the American Philosophical Society,* 2: 11–15.

LEA, I., 1843, Descriptions of new fresh water and land shells. *Transactions of the American Philosophical Society,* 8: 163–250, pl. 5–27.

LEA, I., 1861, Descriptions of a new species of *Neritina* from Coosa River, Alabama. *Proceedings of the Academy of Natural Sciences of Philadelphia,* 13: 55.

LEA, I., 1863, New Melaniidae of the United States. *Journal of the Academy of Natural Sciences of Philadelphia,* 5: 217–356, pl. 34–39.

PILSBRY, H. A., 1906, Note on *Lepyrium. Nautilus,* 20: 51.

PILSBRY, H. A., 1910, New Amnicolidae from the Panuco River system, Mexico. *Nautilus,* 23: 97–100, pl. 9.

PILSBRY, H. A. & OLSSON, A. A., 1951, The Lepyriidae, a new family of fresh-water snails. *Notulae Naturae, Academy of Natural Sciences of Philadelphia,* 233: 1–5.

RADOMAN, P., 1966, The zoogeographic and phylogenetic interrelations of the genera *Lithoglyphus* and *Emmericia. Bulletin du Museum d'Histoire Naturelle du Beograde,* 21: 43–49.

RADOMAN, P., 1973, New classification of fresh and brackish water Prosobranchia from the Balkans and Asia Minor. *Prosebna Izdanja, Museum d'Histoire Naturelle de Beograde,* 32: 1–30.

SAY, T., 1825, Descriptions of some new species of fresh water and land shells of the United States. *Journal of the Academy of Natural Sciences, Philadelphia,* 5: 119–131.

SAY, T., 1829, Descriptions of some terrestrial and fluviatile shells of North America. *New Harmony Disseminator of Useful Knowledge,* 2: 227–230.

STEIN, C. B., 1976, Endangered and threatened plants and animals of Alabama: Gastropoda. *Bulletin Alabama Museum of Natural History,* 2: 21–41.

STIMPSON, W., 1865a, Diagnoses of newly discovered genera of gastropods, belonging to the sub-fam. Hydrobiinae, of the family Rissoidae. *American Journal of Conchology,* 1: 52–54.

STIMPSON, W., 1865b, Researches upon the Hydrobiinae and allied forms. *Smithsonian Miscellaneous Collections,* 201; 1–59.

TAYLOR, D. W., 1966, A remarkable snail fauna from Coahuila, Mexico. *Veliger,* 9: 152–228.

THIELE, J., 1928, Revision des Systems der Hydrobiiden und Melaniiden. *Abteilung für Systematik, Okologie and Geographie der Tiere,* 55: 351–402. pl. 8.

THOMPSON, F. G., 1968, *The aquatic snails of the Family Hydrobiidae of peninsular Florida.* University of Florida Press, Gainesville: i–ix, 1–268.

THOMPSON, F. G., 1969, Some hydrobiid snails from Georgia and Florida. *Quarterly Journal of the Florida Academy of Sciences,* 32: 241–265.

THOMPSON, F. G., 1977, The hydrobiid snail genus *Marstonia. Bulletin of the Florida State Museum,* Biological Sciences, 21: 113–158.

THOMPSON, F. G., 1979, The systematic relationships of the hydrobioid snail genus *Nymphophilus* Taylor, 1966 and the status of the Subfamily Nymphophilinae. *Malacological Review,* 12: 41–49.

TRYON, G. W., 1862, Notes on American fresh water shells, with descriptions of two new species. *Proceedings of the Academy of Natural Sciences of Philadelphia,* 1862: 451–452.

TRYON, G. W., 1870, *A monograph of the freshwater univalve Mollusca of the United States.* Academy of Natural Sciences, Philadelphia: 1–238.

WALKER, B., 1904, New species of *Somatogyrus. Nautilus,* 17: 133–142, 5 pl.

WALKER, B., 1909, New Amnicolidae from Alabama. *Nautilus,* 22: 95-90, pl. 6.

WALKER, B., 1915, Apical characters of *Somatogyrus* with descriptions of three new species. *Nautilus,* 29: 37–41, 49–53.

WALKER, B., 1918, A synopsis of the classification of the fresh-water Mollusca of North America, north of Mexico, and a catalogue of the more recently described species, with notes. *Miscellaneous Publications of the Museum of Zoology, University of Michigan,* 6: 1–213.

WENZ, W., 1938–1939, Gastropoda, Parts 2 and 3, Prosobranchia. p. 432 (*Lepyrium*) and 555–581 (Hydrobiidae) *In* SCHINDEWOLF, O. H., ed. *Handbuch der Paläozoologie.* Borntraeger, Berlin.

APPENDIX A

The following Lithoglyphinae are described. Adequate descriptions of two are not available in contemporary literature. The third species is new, and its anatomy is discussed earlier in this paper.

Lepyrium showalteri (Lea)

Neritina showalteri Lea, 1861: 55.—Lea, 1863: 267, pl. 35, figs. 78, 78a.
Neritella showalteri (Lea), Binney, 1865: 106, fig. 212.
Lepyrium showalteri (Lea), Dall, 1896: 13–15.—Walker, 1918: 38, fig. 139.—Wenz, 1938: 432, fig. 1062.—Pilsbry & Olsson, 1951: 1–5, figs. 1–3, 3a.—Stein, 1976: 25.—Burch & Tottenham, 1980: 104, figs. 192–193.
Lepyrium showalteri cahawbensis Pilsbry, 1906: 51.—Goodrich, 1941: 7, 10.—Pilsbry & Olsson, 1951: figs. 4–6.—Stein, 1976: 25.

Shell (Figs. 59–62). Ovate in outline, adults 3.5–4.4 mm high and 4.0–5.0 mm wide, about 0.81–0.94 times as high as wide. Strongly flattened with a strongly excentric apex and neritid-like in appearance; nearly uniformly dome-shaped with the apex hardly protruding when viewed from the rear (Fig. 61) or front (Fig. 56). Umbilical area imperforate. About 2.3–3.0 whorls, which rapidly expand. Apical whorls usually eroded to the level of the body whorl. Protoconch depressed, nearly smooth with a few low wrinkled depressions along outer surface (Fig. 39). Subsequent whorls rapidly expanding; sculptured with fine growth striations. Peristome greatly expanded, circular, but variable in shape, large specimens tend to have a proportionally higher aperture than do smaller specimens. Columellar callus deeply dished, about a third the width of the aperture area and with a wing-like extension on the upper left corner. Peristome 0.92–1.08 times as high as wide. Width of peristome about 0.79–0.95 time width of shell. Aperture flattened dorso-ventrally, continuing as a spire into upper whorls, not shelf-like as in Neritidae. Measurements in mm of three specimens selected to show variation are:

Cat. no.	Height	Width	Ap. H.	Ap.W.	Whorls
UMMZ 67445	3.84	4.46	3.91	3.60	2.5
UMMZ 67445	4.03	4.65	4.03	4.40	2.7
UMMZ 97448	4.40	4.96	4.40	4.65	3.0

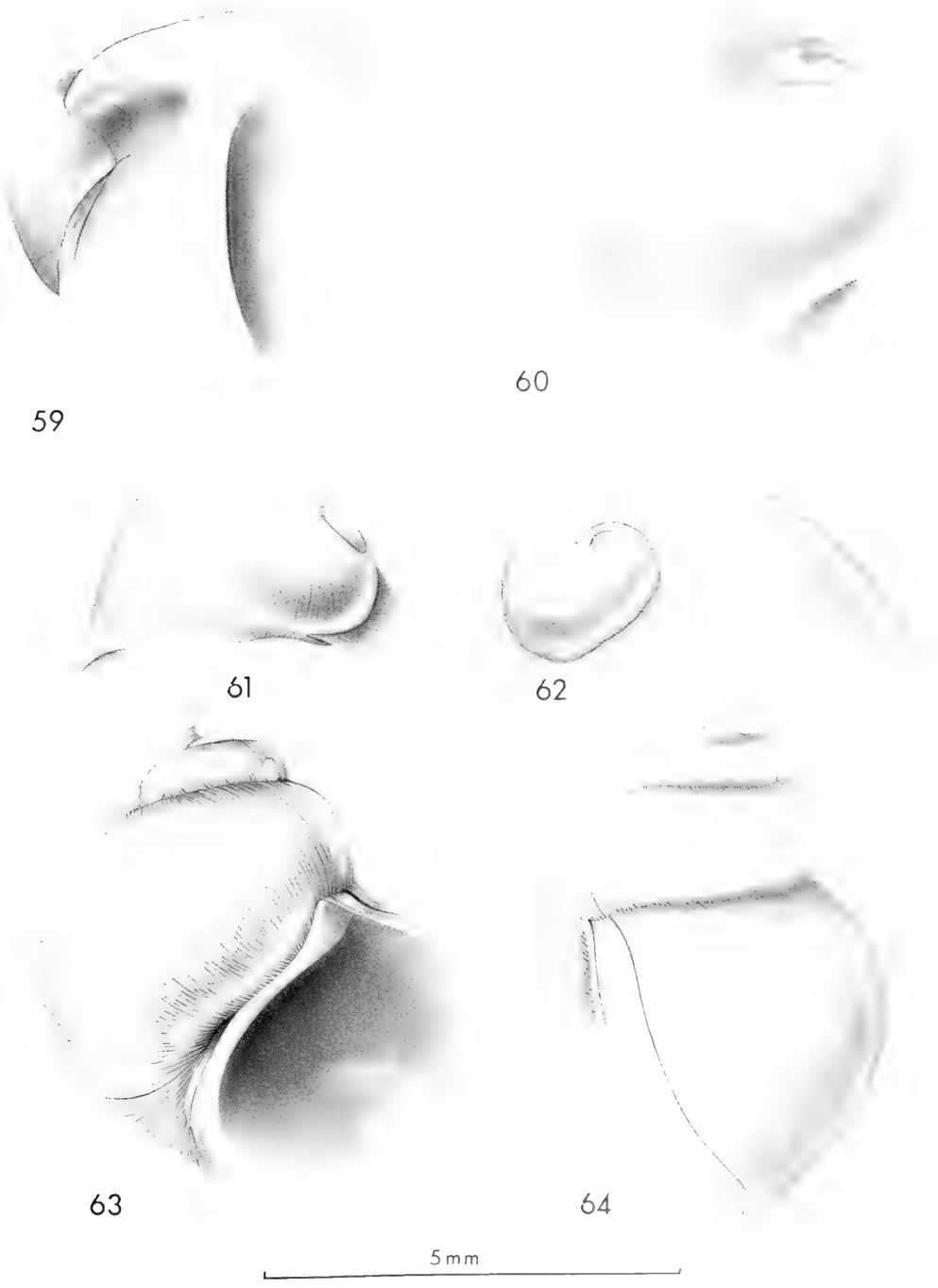
Operculum (Fig. 7). Corneous; retractable into aperture for about ¼ whorl. Paucispiral with the nucleus located close to the lower left margin (neomelanian); 2¼ rapidly expanding whorls. Outer surface sculptured with distinct incremental striations, and finer, close spiral striations.

Distribution. Recorded from a short segment of the Cahaba River and the Little Cahaba River in Bibb County, and a short segment of the Coosa River in Shelby County, Alabama. Presumably it is extinct in the Coosa because of impoundment of the river. It still exists in the Cahaba and Little Cahaba rivers. Records of specimens examined are listed in Appendix C.

Remarks. Pilsbry (1906) recognized two subspecies, the typical subspecies from the Coosa River, and *L. s. cahawbensis* from the Cahaba River. The latter was based upon immature specimens which he characterized as being smaller, with a straight columellar edge, and without a raised outer margin of the columellar area (causing the columella not to be dished). Later Pilsbry & Olsson (1951: 2) stated that the characteristics were inconsistent because they were based on juveniles, and they doubted the validity of *cahawbensis* as a distinct taxon. Unfortunately very little material is available from the Coosa River. However, specimens I have examined demonstrate that only one form is recognizable.

Somatogyrus Gill, 1863

The genus *Somatogyrus* includes 37 described species in North America. There are perhaps half again as many remaining to be described. Most of the described species are poorly known and inadequately illustrated, and must be restudied before meaningful specific comparisons can be made. Useful specific characteristics occur in the pro-



FIGS. 59–64. Figs. 59–62. *Lepyrium showalteri* (Lea), UMMZ 67445: Cahaba River, Guerney, Bibb Co., Alabama. Fig. 63. *Gillia altilis* (Lea), neotype: UF 40550. Fig. 64. *Gillia altilis* (Lea), UF 40551: Lake Waccamaw, Washington Co., North Carolina.

toconch sculpture, the radula, the pigmentation patterns of the mantle, tentacles and snout, the shape and structure of the columellar lip, other aspects of the aperture, size and obesity. Adults of most species are decollate due to erosion of the apical whorls. Thus, the last whorl and the aperture provide the only measurements of height that are useful for specific comparisons. Most species of *Somatogyrus* occur on rocks in high-energy rivers. Some occur in low gradient streams on sand and gravel. Most *Somatogyrus* are annual species. Ovipositing usually takes place in May and June, whereupon the adults die. Morphological maturity of the new progeny occurs in October and November. Most samples that are collected between June and September contain only immature specimens, which have not yet developed the definitive characteristics essential for correct species identifications.

Somatogyrus rheophilus Thompson, new species

Diagnosis. A medium-sized species characterized by the tendency for its whorls to be weakly rounded above the periphery, its narrowly rimate umbilicus, its receded basal lip, its wide, rounded columellar lip, its advanced posterior corner of the aperture, its angular parietal-columellar corner, and its uniform black or grayish-black mantle. It is most similar to *S. alcoviensis* Kreiger from the Yellow River, Newton County, Georgia. The latter differs by having the columellar lip and parietal callus form a weak, oblique arch.

Shell (Fig. 67). Broadly conical or turbinate. Adults decollate with 2–3 whorls remaining. Medium sized for the genus; eroded adults 3.9–4.5 mm high (holotype 4.3 mm); body whorl about 3.5–4.0 mm high and 3.4–4.0 mm wide. Width about equal to height of last whorl (0.97–1.02). Penultimate whorl 0.48–0.54 times width of last whorl. Last whorl nearly flattened above periphery. Suture weakly impressed. Umbilicus narrowly rimate, or occasionally imperforate. Periostracum yellow-green with oblique, shallow growth striations. Protoconch (Fig. 40) with fine spiral threads at and below periphery; with dimples superimposed on spiral threads above periphery. Aperture broadly ovate, 0.83–0.95 times as high as wide, 0.66–0.73 times height of last whorl. Plane of aperture at 22–30° to axis of shell. Peristome complete across parietal area by a thick callus. Basal lip slightly receded; base-columellar corner projecting forward. Posterior corner of aperture advanced forward and with a shallow angular groove internally. Columellar lip very wide; rounded in cross section; straight or weakly concave in lateral profile; vertical. Columellar lip forming a pronounced angle with parietal callus.

Measurements in mm for the holotype and ten paratypes selected to show variation follow (holotype in parenthesis).

Height, 3.5–4.0 (3.9); width, 3.4–4.0 (3.8); aperture height, 2.4–2.8 (2.8); aperture width, 2.0–2.5 (2.4).

Type-locality. Georgia, Upson County, Flint River at Spewrell Bluff, U.S. Army Corps of Engineers river mile 200. Holotype: UF 40500; collected 22 May 1981 by Fred G. Thompson. Paratypes: UF 31244 (246 specimens); 25 specimens each deposited in ANSP, FMNH, MCZ, UMMZ, USNM, Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands, Senckenbergische Naturforschende Gesellschaft, Frankfurt-am-Main, Germany, and Herbert D. Athearn collection; same data as holotype.

Distribution. Endemic to the middle section of the Flint River in Georgia from Meriwether-Pike counties southeast to Taylor-Crawford counties. This species has been found only in shoals and rapids where it occurs on granite boulders and gravel in moderate currents. Locality records are given in Appendix C.

Gillia altilis (Lea)

Melania altilis Lea, 1841: 13.—Lea, 1843: pl. 5, figs. 23.

Leptoxis altilis (Lea), Haldeman, 1847: 6, pl. 5, fig. 152.

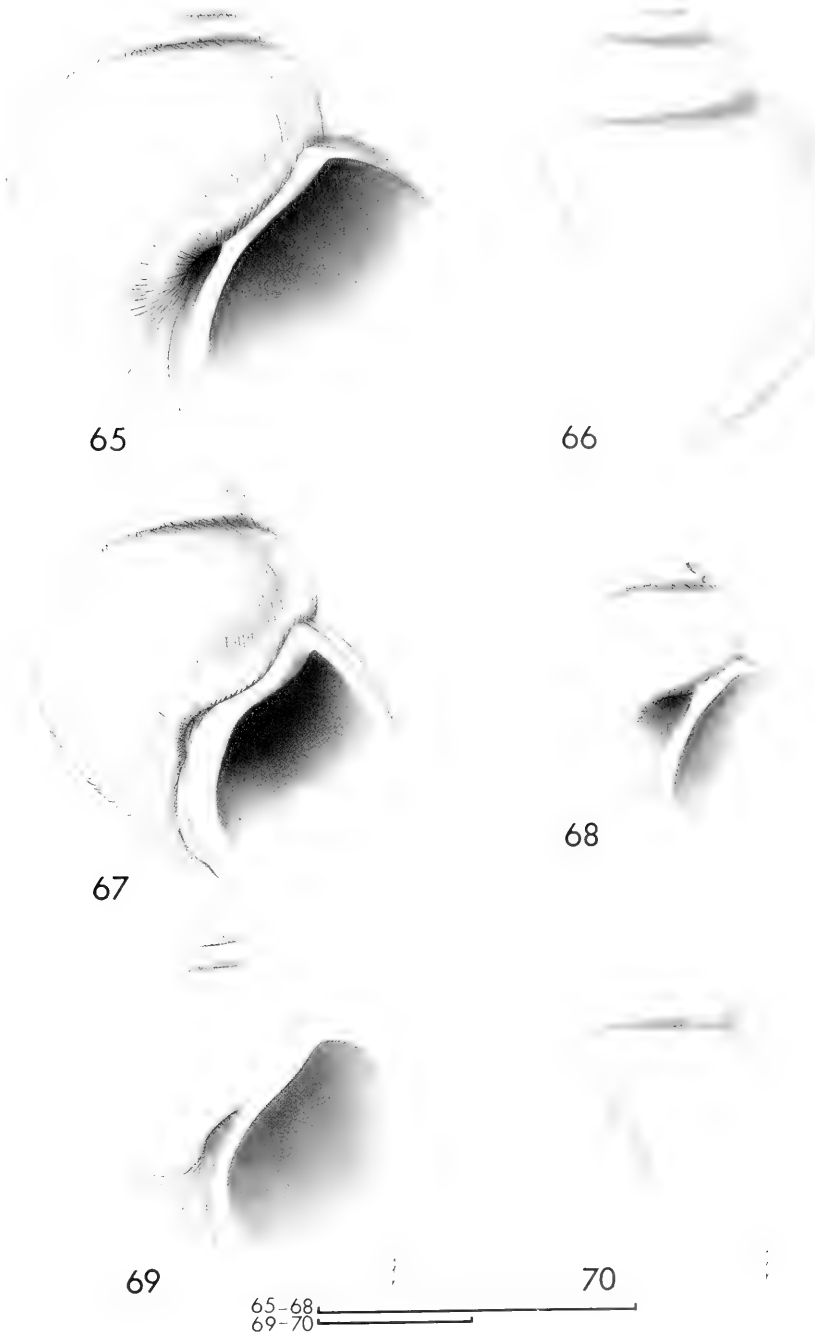
Gillia altilis (Lea), Stimpson, 1865a: 53.—Stimpson, 1865b: 51.—Binney, 1865: 74–75, fig. 146.—Walker, 1918: 32–33, figs. 115–116.—Burch & Tottenham, 1980: 104, fig. 191.—Burch, 1982: 23, fig. 191.

Somatogyrus altilis (Lea), Tryon, 1870: 60, pl. 17, fig. 9.

Leptoxis crenata Haldeman, 1847: 6, pl. 5, fig. 153.

Gillia crenata (Haldeman), Binney, 1865: 74–75, figs. 147–148.

Shell (Figs. 63, 64). Conico-globose. Light yellow-green. About 4.5 whorls, but apex usually eroded, leaving 2–4 whorls in adults. Moderately large, eroded adults usually 6–8 mm high (lectotype 6.6 mm). Body whorl



FIGS. 65–70. Figs. 65–66. *Somatogyrus depressus* (Tryon), UF 34969: Mississippi River, Davenport, Iowa. Fig. 67. *Somatogyrus rheophilus* n. sp.; holotype: UF 40500. Fig. 68a. *Clappia umbilicata* (Walker); ANSP 95037: Coosa River, Alabama. Figs.69–70. *Birgella subglobosa* (Say); UF 35008: Ohio River at Five Mile Creek, Hamilton Co., Ohio.

conspicuously enlarged; adults tending to be shouldered or fluted below suture; height of body whorl 0.95–1.10 times width. Shell usually rimate; some specimens imperforate, or narrowly umbilicate. Apical whorl of protoconch elevated, 1.25 mm wide transverse to initial suture. Protoconch sculptured with fine spiral threads that are uniformly dispersed over surface of first whorl (Fig. 41). Subsequent whorls strongly rounded; sculptured with distinct, regularly spaced incremental striations; usually with 1–2 dark growth varices. Aperture broadly ovate-auriculate in shape. Plane of aperture lying at an angle of 18–20° to axis of shell. Height of aperture 0.70–0.78 times height of body whorl; about 0.78–0.90 times as wide as high. Peristome in mature specimens complete across parietal wall by a thin callus; incomplete in sub-adults; peristome dark rimmed. Columellar lip moderately thickened, rounded. Outer lip and basal lip sharp-edged, evenly curved through columella. Outer lip conspicuously arched forward in lateral profile (Fig. 64).

Operculum (Fig. 52) oval in shape. Chitinous, yellowish-green. Paucispiral, consisting of three whorls. Nucleus located in the lower left third. Outer surface sculptured with fine incremental striations.

Measurements in mm based on 29 specimens selected to show variation are given below. UF 27500—Lake Waccamaw, North Carolina; UF 35027—Potomac River, District of Columbia; UF 35013—Erie Canal, New York.

Cat. no.	n	Total h.	Body wh. h.	Width	Apert. h.	Apert. w.
UF 27550	10	5.6–5.9	5.4–6.1	5.3–5.8	4.0–4.5	3.5–3.9
UF 35025	8	6.5–8.1	5.5–7.4	5.3–7.6	3.9–5.5	3.5–4.5
UF 35013	10	5.6–7.0	5.0–6.1	4.9–6.1	3.5–4.6	3.0–3.9
UF 40550	neotype	6.6	5.9	5.6	4.3	3.9

Type-locality. Lea (1841) stated that his specimens of *Melania altilis* came from the Santee Canal, South Carolina, and the Susquehanna River at Havre de Grace, Maryland. Haldeman (1847) stated that the type-specimen of *Leptoxis crenata* came from the Santee Canal, South Carolina. Type-specimens for neither *Melania altilis* Lea nor *Leptoxis crenata* Haldeman can be located. Presumably they are lost. It is clear that they are the same species, and a neotype must be designated. *Melania altilis* Lea: Neotype UF 40550 (Fig. 63). *Leptoxis crenata* Haldeman: Neotype UF 40550; same specimen as neotype for *Melania altilis* Lea. Neotype locality: Lake Waccamaw, Columbus County, North Carolina; neotype collected 12 September 1980 by Fred G. Thompson. Lake Waccamaw is selected as the neotype locality for the following three reasons. The species shows little variation throughout its range, and there is no basis to suspect that the Lake Waccamaw population is different from other populations in its taxonomic identity. On three occasions in 1980, 1981, and 1982, I was unsuccessful in finding the species in the Santee River. Apparently it no longer occurs there. The anatomical data given in this paper are based on Lake Waccamaw material.

Distribution (Fig. 71). The species is widely distributed in rivers draining into the Atlantic Ocean from South Carolina north to New York and Vermont. It has entered the Lake Ontario system *via* the Erie Canal. The species is found in quiet lakes and rivers, as well as fast gradient streams. Locality records for this species are given in Appendix C.

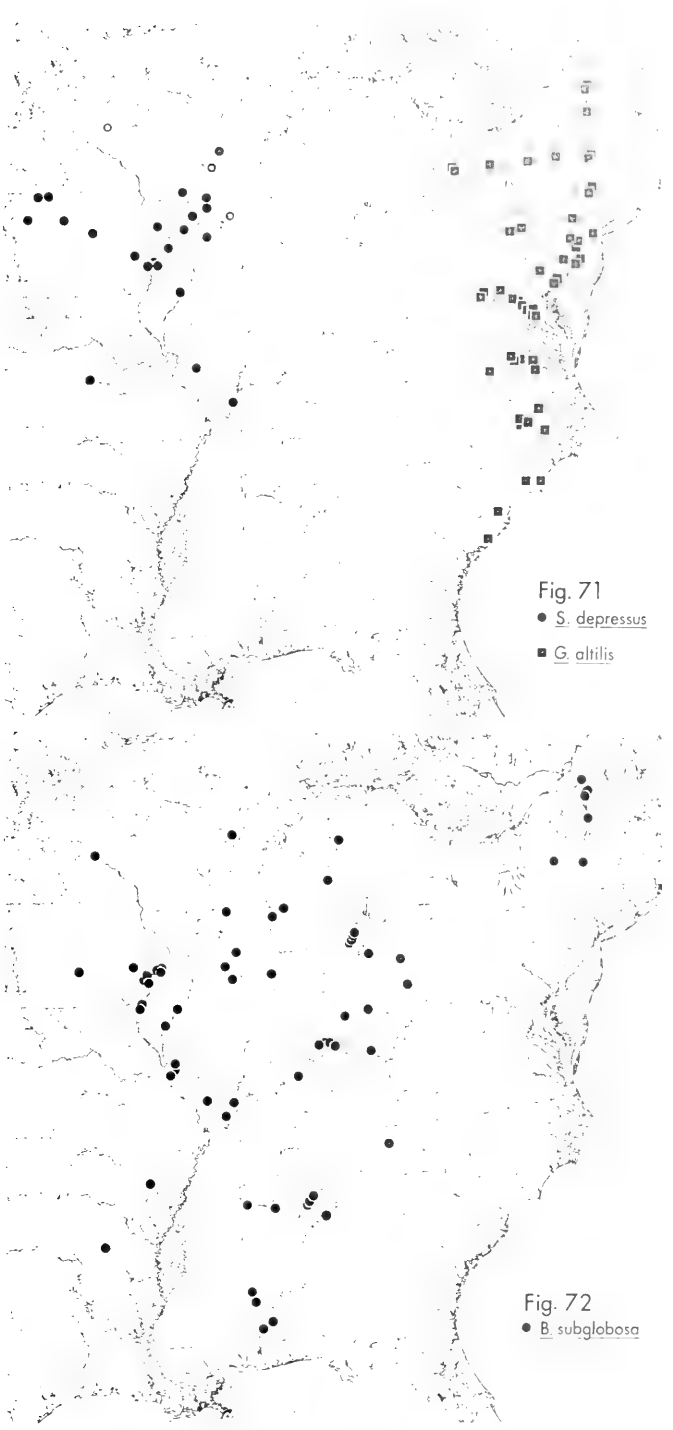
APPENDIX B

A North American snail incorrectly associated with the Lithoglyphinae:

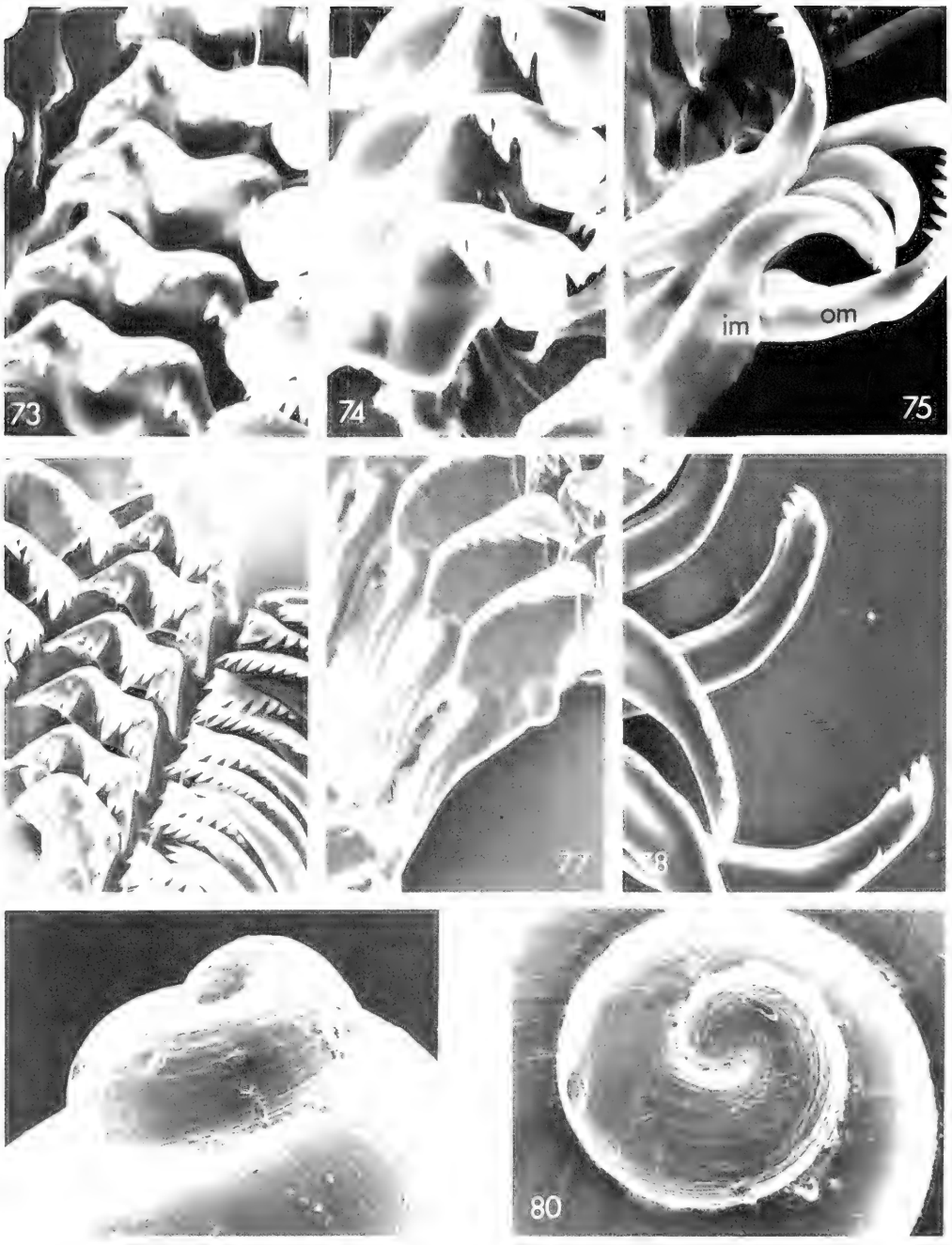
Birgella Baker, 1926

Birgella Baker, 1926: 196.—Baker, 1928: 154–155.—Wenz, 1939: 575.—Thompson, 1979: 47.—Burch & Tottenham, 1980: 110. (Type-species: *Paludina subglobosa* Say, 1825).

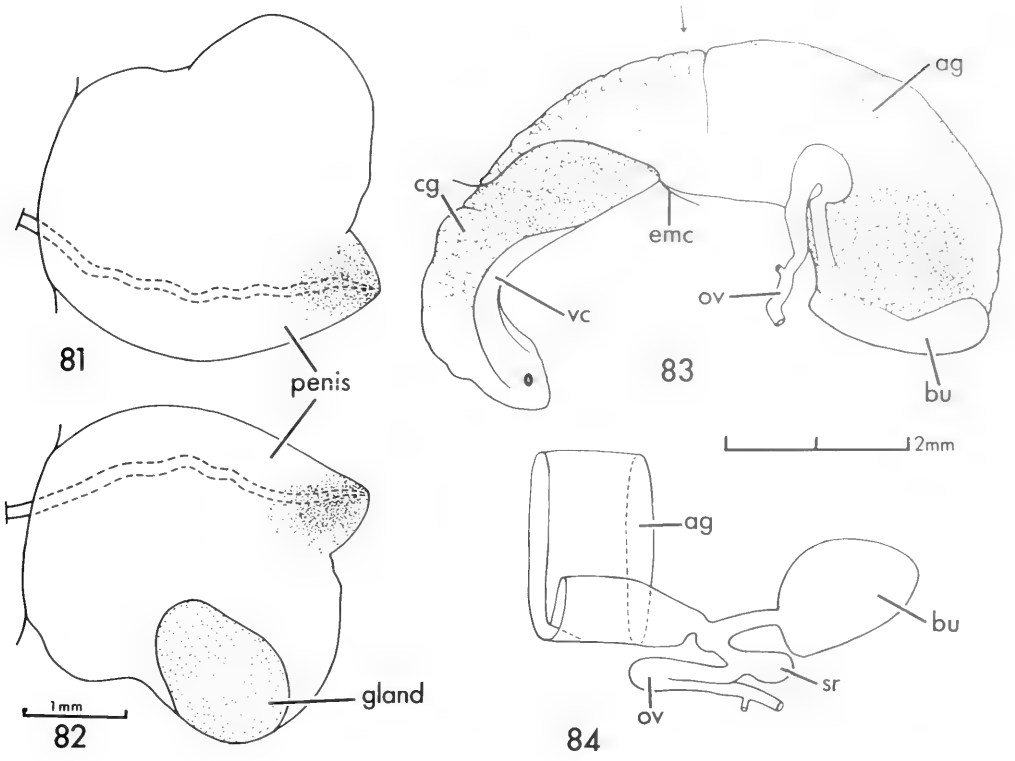
The shells of *B. subglobosa* (Say) (Figs. 69–70) are so similar to those of *Somatogyrus depressus* (Figs. 65–66), the type-species of *Somatogyrus*, that they were considered congeners for more than a century. Baker (1928) separated *Birgella* from *Somatogyrus* on the basis of differences in the verge and radula, but placed both genera in the Lithoglyphinae. Most subsequent authors failed to recognize *Birgella* as a distinct genus due to



FIGS. 71–72. Geographic distributions. Fig. 71. *Gillia altilis* (Lea) and *Somatogyrus depressus* (Tryon). Fig. 72. *Birgella subglobosa* (Say).



FIGS. 73–80. SEM photographs of radulae and protoconch sculpture. Figs. 73–75. *Nymphophilus minkleyi* Taylor, UF 34905: creek 10 km SW Cuatro Cienegas, Coahuila, Mexico. Figs.76–78. *Birgella subglobosa* (Say), UF 35275, Alabama River, Choctaw Bluff, Clarke Co., Alabama. Figs. 79–80. *Birgella subglobosa* (Say), UF 35277: Ohio River at Five Mile Creek, Hamilton Co., Ohio. Enlargements: Figs. 73–75, 78 ×356. Figs. 76, 77 ×238. Figs. 78–80 ×48.



FIGS. 81–84. *Birgella subglobosa* (Say). Figs. 81–82. Verge. Fig. 83. Female reproductive system without ovary. Fig. 84. Mid-segment of oviduct showing relationships between seminal receptacle, bursa copulatrix, and albumen gland. Legend: ag = albumen gland; bu = bursa copulatrix; cg = capsule gland; emc = posterior wall of mantle cavity; ov = oviduct; sr = seminal receptacle.

the limited anatomical data available on the Hydrobiidae. Thompson (1979) placed *Birgella*) in the Nymphophilinae, where it is clearly related because of features of the reproductive system and radula. Clarke (1981) continued to treat *Birgella* as a synonym of *Somatogyrus*. *Birgella* is differentiated from other nymphophilids because of its protoconch sculpture, its large globose shell and its ponderous penis. It is not closely related to other known genera. The protoconch sculpture is vaguely similar to that of *Nymphophilus* because of its coarse step-like wrinkles (see Thompson, 1979). The morphology of the penis has some similarities to that of *Marstonia*. Both have a single apocrine gland confined to the apical lobe (see Thompson, 1977, for a discussion of the morphology of *Marstonia*). The two genera have such dissimilarly shaped penises, the apocrine gland pattern must be considered convergent. *Birgella* is characterized within the Nymphophilinae as follows.

Shell (Figs. 69–70). Large, about 6–9 mm high with about 4.3 whorls; globose, usually about 0.83–0.87 times as wide as high; some specimens as wide as high with a very ample aperture and a depressed spire. Protoconch sculptured with step-like, rugose wrinkles with superimposed spiral threads (Fig. 79–80). The spiral threads are a unique feature within the subfamily. Operculum paucispiral with about 2.5 whorls.

Penis (Figs. 81–82). With a large globose apical lobe on the left side and a short stocky tip on the right side. Tip pigmented with melanophores. Apical lobe with a large circular apocrine gland on the inner surface (incorrectly reported to be absent in an earlier report (Thompson, 1979)).

Female reproductive system (Figs. 83–84). Typical for subfamily Nymphophilinae (see

Thompson, 1979). A single seminal receptacle present and completely buried in albumen gland. Ventral canal of anterior pallial oviduct spirally offset. Lumen of canal continuous with capsule gland lumen (Fig. 84).

Radula (Figs. 76–78). With large acuminate cusps. Central tooth with 2–3 basocones on each side located on a reflected lateral ridge. Lateral teeth as in other Nymphophilinae, with a rounded basal lobe. Radular data for two specimens I examined (UF 35275, 35278) are given in Table 1. Baker (1928) and Berry (1943) gave slightly different cusp counts.

The radula of *Nymphophilus minkleyi* Taylor is figured for comparison (Figs. 73–75). *Birgella* contains a single species.

Birgella subglobosa (Say)

Paludina subglobosa Say, 1825: 125.—Haldeman, 1847: 10–11, pl. 10, fig. 7 (Type-locality: Northwestern Territory).
Somatogyrus subglobosus (Say), Tryon, 1870: 60–61, pl. 17, figs. 10–11.—Baker, 1902: 340–341, fig. 123.—Berry, 1943: 49–52, pl. 2, fig. 1 (shell), pl. 4, fig. 3 (radula), text-fig. 8 (penis).
Melania isogona Say, 1829: 277. (Type-locality: Bear Grass Creek, near Louisville, Kentucky).
Somatogyrus isogona (Say), Stimpson, 1865b: 22.—Binney, 1865: 77–78, fig. 151.
Birgella s. subglobosa (Say), Baker, 1928: 155–158.—Burch & Tottenham, 1980: 110, figs. 188, 198, 202.
Birgella subglobosa isogona (Say), Baker, 1928: 159–161, pl. 8, figs. 10–12.
Paludina pallida Lea, 1839: 22, pl. 23, fig. 104. (Type-locality: near Cincinnati, Ohio).

This is a well-known North American species. Detailed descriptions are given in Baker (1928) and Berry (1943). Some authors recognize two subspecies. The typical subspecies is said to be narrowly umbilicate or rimate and to have a thin parietal callus. The other subspecies is said to be imperforate and to have a thicker parietal callus (Baker, 1928). The material I have examined shows that these characters vary throughout the range of the species, and that they do not segregate with any geographic or ecological factor. Indeed, the differences seem to be consequences of growth. Old specimens have a thicker callus which tends to cover the umbilicus. Thus the two forms do not meet the accepted criteria for subspecies.

Birgella subglobosa is widely distributed in the central United States from Wisconsin, Michigan, and Ohio south to Arkansas and Alabama (Fig. 72). It also occurs in New York in the Mohawk and Hudson River systems. Usually it is found in large rivers and lakes. It is not confined to deep water, contrary to published statements. I have collected it in bays and sloughs at less than 1 meter depth. The snail is found most commonly in quiet water on a soft silt substrate. Specimens examined are listed in Appendix C.

APPENDIX C

Specimens of HYDROBIIDAE examined during this study are from the following museum collections, which are designated as indicated in parenthesis: Academy of Natural Sciences, Philadelphia (ANSP), Carnegie Museum, Pittsburgh (CM), Field Museum of Natural History (FMNH), Museum of Comparative Zoology, Harvard University (MCZ), Florida State Museum, University of Florida (UF), Museum of Zoology, University of Michigan (UMMZ), National Museum of Natural History (USNM).

Lepyrium showalteri (Lea)

ALABAMA.—*Bibb Co.* Cahaba River (UMMZ 97447, UMMZ 97445, MCZ 133133); Cahaba River, near Anita (UMMZ 68301); Cahaba River, 1.6 km N. Centerville (USNM 672419, MCZ 252247); Cahaba River, Lilly Shoals (UMMZ 49272); Cahaba River, near Piper (MCZ 99174, UMMZ 69886, UMMZ 65996); Little Cahaba River (MCZ 99175, UMMZ 97444, UMMZ 97448); Little Cahaba River, 4.8 km E Piper (UMMZ 67444). *Dallas Co.*: Cahaba River Wildcat Island, near Cane Creek (UMMZ 87446). *Shelby Co.*: Coosa River (USNM 29016); Gurnee (MCZ 299176, USNM 321180, 321181); Coosa River, 16.1 km above Ft. Williams (USNM 102851, lectotype by present designation; USNM 102851a). Fort Williams was at the confluence of the Coosa River and Cedar Creek, W. of Fayetteville, Talladega Co., Alabama.

Clappia clappi Walker
(= *Clappia umbilicata* Walker)

ALABAMA.—*Chilton Co.*: Coosa River, Duncans Riffle (ANSP 95307, paratypes).

Somatogyrus depressus (Tryon)

ILLINOIS.—*DeKalb Co.*: Kishwawkee Creek (MCZ 46592). *Fulton Co.*: Canton (UMMZ 116934, UF 34975). *Hardin Co.*: Elizabethtown, Ohio River (UMMZ 49781, UF 34976). *Rock Island Co.*: Rock Island (USNM 476878, USNM 512075). *Stephenson Co.*: 3.2 km S. Freeport (USNM 49781). *Washington Co.*: Okaw River, Covington (MCZ 68437).

IOWA.—*Cherokee Co.*: Cherokee (USNM 507933). *Clinton Co.*: Clinton (USNM 539849). *Dickinson Co.*: NE Okoboji (USNM 667011). *Dubuque Co.*: Dubuque (UMMZ 143740). *Emmet Co.*: Estherville (USNM 506130); Des Moines River near Estherville (USNM 526533). *Hardin Co.*: Eldora (USNM 506379, USNM 519429, USNM 514807); Iowa River, Eldora (FMNH 130351). *Humboldt Co.*: Dakota City (USNM 526532). *Johnson Co.*: Iowa City (USNM 506380, UMMZ 69940). *Muscatine Co.*: Muscatine (USNM 508369). *Scott Co.*: Davenport (USNM 121041, USNM 27904, USNM 38414—SEM radula, UF 34968–34970).

MISSOURI.—*Benton Co.*: Warsaw, Osage River (UMMZ 67435, UF 34980).

WISCONSIN.—*Brown Co.*: De Pere (FMNH 10649). *Jefferson Co.*: Pipersville Rapids, Bark River (UMMZ 116940, UF 34979); Watertown (UMMZ 143742, UF 34977). *Rock Co.*: Evansville (CM 62.24127). *Sauk Co.*: Wisconsin River (UMMZ 143743, UF 34981); Prairie du Sac (UF 34978).

Somatogyrus rheophilus Thompson

GEORGIA.—*Meriwether Co.*: Flint River, 2.7 km NE Gay (UF 40508); Flint River, 2.1 km E Gay (UF 40502); Flint River, 5.6 km SE Gay (UF 40503, 40506). *Talbot Co.*: Flint River, 5.1 km NW Carsonville (UF 40501); Flint River, 3.5 km NNW Fickling Mill (UF 40509). *Taylor Co.*: Flint River, 5.8 km NW Fickling Mill (UF 40507). *Upson Co.*: Flint River at Spewrell Bluff (type-series); Flin River, 11.9 km WSW Thomaston (UF 40504); Flint River at Yellowjacket Shoals, 9.7 km SW Thomaston (UF 31241); Flint River, 9.7 km SW Lincoln Park (UF 34902, SEM shell; UF 34903, SEM radula; UF 40511); Flint River, 11.3 km SSW Lincoln Park.

Gillia altilis (Lea)

DISTRICT OF COLUMBIA.—*Arlington Co.*: Analston (USNM 336089, USNM 252023); Anacostia River, Buzzard's Point (USNM 697026); Popular Point (USNM 697025); Analston Id. (USNM 697015); between P.R.R. Bridge and Pa. Ave. Bridge (USNM 697021); C & O Canal (USNM 335872); near Asylum Wharf, East Branch (USNM 697023); Potomac River (CM 62.24224); above Long Bridge (CM 62.25465); Fox Ferry (USNM 251542, USNM 271700, USNM 465805, MCZ 2181, UMMZ 28918); E. Branch, Potomac River (UF 35025); Potomac River (UF 35024, UF 1002).

MARYLAND.—*Allegany Co.*: Cumberland (USNM 149952); Fall of the Potomac, Potomac State Forest (MCZ). *Cecil Co.*: near Charlestown (USNM 521817). *Hartford Co.*: Havre de Grace (USNM 121450). *Montgomery Co.*: Cabin John, C & O Canal (MCZ 2179); Sycamore Island (USNM 521974). *Prince Georges Co.*: Fort Washington (UMMZ 118414, UMMZ 364722); Fort Washington, Potomac River (USNM 227686); Fort Washington, Piscataway Creek (UF 35021).

NEW JERSEY.—*Burlington Co.*: Burlington (USNM 120468); Burlington, Delaware River (MCZ 57089). *Essex Co.*: Newark, Morris Canal (MCZ 186744). *Hunterson Co.*: Lambertville (USNM 536807). *Mercer Co.*: Raritan Canal, aqueduct near Princeton (CM 62.5699). *Sussex Co.*: Flatbrookville (MCZ 75217). *Warren Co.*: Phillipsburg, Delaware River (FMNH 87953).

NEW YORK.—*Albany Co.*: Albany (CM): Albany, Hudson River (USNM 465755, FMNH 59965, UF 35015). *Dutchess Co.*: Tivoli, Hudson River (MCZ). *Herkimer Co.*: Mohawk, Erie Canal (UMMZ 45998, UF 35013, USNM 697027, USNM 697028, UMMZ). *Monroe Co.*: Brighton (UMMZ 118415, UF 35014). *Niagara Co.*: Niagara Falls (USNM 473979). *Onondaga Co.*: Syracuse, Erie Canal (UMMZ 69880, UF 35019, FMNH 58688, UMMZ 69880). *Rensselaer Co.*: Troy (UMMZ 118412, UF 35012); Troy, Champlain Canal (MCZ 2178). *Ulster Co.*: Heath, Hudson River (MCZ 186739). *Wayne Co.*: Clyde (USNM 597809).

NORTH CAROLINA.—*Columbus Co.*: Lake Waccamaw (UF 28439, UF 29652, UF 28077, UF 29637, UF 29644, UF 27550, UF 35044, UF 34901—SEM shell, UF 34816—SEM radula). *Edgecombe Co.*: Swift Creek at NC Hwy. 97 (UF). *Nash Co.*: Tar River, S of Moccasin Creek (UMMZ 197725). *New Hanover Co.*: Wilmington, Greenfield Pond (UMMZ 69881). *Pitt Co.*: Little Continea River, 9.7 km SE Farmville (UMMZ 197724); S of Sandy Cross (UMMZ).

ONTARIO.—*Lincoln Co.*: Niagara-on-the-Lake (MCZ 104863).

PENNSYLVANIA.—*Bucks Co.*: Delaware River, New Hope (CM 62.5700). *Clinton Co.*: Flemington (USNM 28102). *Chester Co.*: Schuylkill River, Phoenixville (FNMH 87956). *Lancaster Co.*: Columbia (CM 62.16370, MCZ 2180, MCZ 186745). *Lycoming Co.*: Muncy, canal (MCZ). *Northampton Co.*: Delaware River, Easton (FMNH 15693, UF 35020). *Philadelphia Co.*: Philadelphia (FMNH 87950); Schuylkill Canal, Manayunk (CM 62.5701); Philadelphia (MCZ 186743).

SOUTH CAROLINA.—*Charleston Co.*: Charleston (MCZ). *Williamsburg Co.*: Lynche's Creek (USNM 63973).

VERMONT.—*Franklin Co.*: St. Albans Bay (CM 62.32653). *Grand Isle Co.*: Grand Isle, Lake Champlain (UMMZ 118422, UF 35018); Lake Champlain, Chimney Point (USNM 336443, USNM 336442, USNM 336445, USNM 336444, USNM 591730).

VIRGINIA.—*Alexandria Co.*: Potomac River (MCZ 186741, MCZ 70540). *Amherst Co.*: James River, Lynchburg (USNM 451904). *Cumberland Co.*: Cartersville, James River (MCZ 261289). *Fairfax Co.*: Dyke, near Mt. Vernon (USNM 420546); Mt. Vernon (UF 18435); near Great Falls (numerous lots, USNM, MCZ, UMMZ, UF). *Goochland Co.*: Columbia, James River (MCZ 261334). *Henrico Co.*: Richmond, James River (CM 62.24126). *Loudoun Co.*: Potomac River, 6.4 km N. Seneca, MD (USNM 697018). *Powhatten Co.*: James River across from Maidens (MCZ 261307). *Prince Co.*: Petersburg (FMNH 87949, USNM 121477).

WEST VIRGINIA.—*Jefferson Co.*: Harper's Ferry (MCZ 136500); Harper's Ferry, Potomac River (MCZ). *Morgan Co.*: Cherry Run, Potomac River (numerous lots UMMZ, MCZ, FMNH, UF).

Fluminicola nuttalliana (Lea)

OREGON.—*Lane Co.*: Willamette River, Eugene (UF 40521). *Linn Co.*: Willamette River, Albany (UF 40523, UF 40524, SEM shell).

Birgella subglobosa (Say)

ALABAMA.—*Choctaw Co.*: Tombigbee River, Ezell Fish Camp, E. of Lavaca (UF 35093). *Clarke Co.*: Alabama River, Choctaw Bluff (CM 65-57). *Colbert Co.*: Tennessee River, Mile 261.0, Union Carbide (UF 34998). *Limestone Co.*: Tennessee River, Mile 291.76. Brown's Ferry (UF 35001); Tennessee River, Mile 288.78, Brown's Ferry (UF 34999). *Monroe Co.*: Alabama River, Clairborne (UF 35099). *Sumter Co.*: Tombigbee River, Lock #3, ESE of Whitfield (UF 35095).

ARKANSAS.—*Dallas Co.*: Ouachita River, 3.9 km W. Sparkman (UF 35002). *Jackson Co.*: White River, Newport (MCZ 66659).

GEORGIA.—*Floyd Co.*: Silver Creek (UF 40639).

ILLINOIS.—*Cass Co.*: Beardstown, Illinois River (UMMZ 197758, FMNH 15694). *Cook Co.*: Chicago, Lake Michigan (FMNH 71888). *Fulton Co.*: Canton (FMNH 71890). *Gallatin Co.*: Shawneetown (FMNH 115352). *Kankakee Co.*: Kankakee Feeder (FMNH 58685). *Madison Co.*: Alton, Mississippi River (UMMZ 197760). *Mercer Co.*: Mississippi River (UMMZ 143748, MCZ 2202); Myers Slough (MCZ 2201). *Pope Co.*: Golconda, Ohio River (UMMZ 197755). *Rock Island Co.*: Moline, Mississippi River (USNM 465760). *Will Co.*: Dupage River, Joliet (FMNH 58686); Joliet (CM 62.25453). *Williamson Co.*: Blaireville, Big Muddy River (UMMZ 117191).

INDIANA.—*Dearborn Co.*: Ohio River, Lawrenceburg (FMNH 87928). *Floyd Co.*: (FMNH 58687). *Marshall Co.*: Lake Maxinkuckee (USNM 697009).

IOWA.—*Johnson Co.*: (FMNH 58701); Iowa City (UMMZ 143749). *Lee Co.*: Mississippi River, 4.7 km N Keokuk (UF 35009); Keokuk, pool above dam (MCZ 175918); Ft. Madison (UF 34997); Montrose (UF 35006). *Muscatine Co.*: Keokuk Lake (USNM 600748); Muscatine, Mississippi River (UF 31307). *Polk Co.*: Des Moines, Des Moines River (MCZ 2196); Des Moines, Bayou at N end Fort Dodge (UF 35011). *Scott Co.*: Le Clair, Mississippi River (MCZ 2197); Mississippi River, Davenport (UF 35003).

KENTUCKY.—*Campbell Co.*: mouth of Five-Mile Creek (UMMZ 70020). *Jefferson Co.*: Louisville, Falls of the Ohio (FMNH 87919).

MINNESOTA.—*Washington Co.*: Ft. Snelling, Minnesota River (MCZ 2200).

MISSOURI.—*St. Louis Co.*: Mississippi River near White House (UMMZ 177032); Jefferson Barracks (UMMZ 197756); Kirkwood, Meramec River (UMMZ 197759).

NEW YORK.—*Herkimer Co.*: Mohawk (FMNH 15520); Mohawk, Erie Canal (MCZ 62233); Mohawk, Mohawk River (CM 62.7056). *Schenectady Co.*: Schenectady (MCZ).

OHIO.—*Erie Co.*: Sandusky, Lake Erie

(CM 62.25454). *Franklin Co.*: Columbus (USNM 30139); Columbus, Ohio Canal (CM 62.7057). *Green Co.*: Clifton, Miami Canal (USNM 28515). *Hamilton Co.*: Cincinnati, (FMNH 115552); Cincinnati, Ohio River (CM 62.25457); Culloms Riffle, Ohio River (FMNH 87924); Five-Mile Creek, Ohio River (FMNH 87922); Mouth of Great Miami River (FMNH 87913); old canal bed near Harrison (FMNH 87912); 9.7 km W. Cincinnati (UMMZ 45448). *Scioto Co.*: Portsmouth, Ohio River (CM 62.8229). *Summit Co.*: (UF 35010). *Tuscarawas Co.*: Mill Race on Ohio Canal, New Philadelphia (CM 62.26560); Tuscarawas River, New Philadelphia (CM 62.25456).

QUEBEC.—*Rouville Co.*: Richelieu River, 3.2 km S. Iberville (MCZ).

TENNESSEE.—Nolachucky River (UF 35007).

VERMONT.—*Addison Co.*: Chimmey Point, Lake Champlain (MCZ 28232); Hospital Creek (USNM 336445). *St. Franklin Co.*: Lake Champlain, St. Albans Bay (MCZ 142046). *Grand Isle Co.*: Lake Champlain, Grand Isle, 3.2 km SE Hero (MCZ).

VIRGINIA.—*Fairfax Co.*: Great Falls (USNM 252381) (doubtful record).

WISCONSIN.—*Milwaukee Co.*: Milwaukee (MCZ).

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IMPLICATIONS OF RADULAR TOOTH-ROW FUNCTIONAL INTEGRATION FOR ARCHAEOGASTROPOD SYSTEMATICS

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ABSTRACT

Functional analysis of complex morphology can be used to generate new sets of taxonomic characters. It may also suggest ecological or biomechanical relationships among characters that otherwise might appear adaptively unrelated. Analysis of tooth-row functional integration in the rhipidoglossan archaeogastropod radula reveals mechanically and taxonomically distinctive interactions between tooth bases, shafts, and cusps. Many of these interactions follow a complex sequence that is not related to the standard concept of teeth arranged in rows and columns. In some groups, rows are impossible to identify in a functional sense, and the concept of base rows and cusp rows is introduced to help clarify previously unappreciated aspects of mechanical integration.

Drawing phylogenetic inferences from functional characters carries the burden of demonstrating that convergence has not occurred. In rhipidoglossan radulae the characters involved in integrating tooth-row function are superimposed on conserved groundplans. Even where two morphological solutions to the same functional problem are extremely similar, traces of underlying phylogenetic differences remain.

Adaptations of cusps for transmitting forces from one tooth to another are taxonomically distinctive as are the shaft expansion and interleaving patterns that transmit force in different ways in different taxa. Basal interaction patterns are particularly elegant because they perform many mechanical functions simultaneously. The lateromarginal plate, a tooth base that is modified as an articulatory structure, has developed independently in different rhipidoglossan groups and is particularly useful in evaluating phylogenetic relationships.

INTRODUCTION

Taxonomists traditionally have plied their trade with tools for assessing the numbers, sizes, shapes, and qualities of morphology that are best observed in static, nonfunctioning (usually dead) organisms. Function and principles of design and construction frequently are tied to character convergences and are such poor indicators of evolutionary relationships at a superficial level that many systematists have avoided functional characters in classification and phylogenetic inference.

And yet superficially similar morphologies may have subtle but profoundly different underlying designs that reflect quite different phylogenetic heritages. Such differences may be more obvious in living, functioning organisms. Functional studies can provide valuable clues for reassessment of morphology and for generating new and more refined sets of taxonomic characters.

For the molluscan taxonomist, the radula is an excellent example of a complex morphological apparatus that does not really have much of a story to tell when only numbers, sizes, and shapes are considered. Radulae have been used extensively in molluscan classification for many years; but their use has, on the whole, been uncreative. The vocabulary for describing radulae is appallingly depauperate, particularly with respect to shape descriptors, in light of the incredible complexity and array of morphologies that have developed within the phylum.

One way to circumvent the vocabulary problem with complex morphology is simply to illustrate it. "Here is the *Pleurotomaria* radula, and a picture is worth a thousand words." Other alternatives, which have not yet come of age, are computer-assisted multivariate morphometric analysis and computer graphic analysis from scanning electron stereo-micrograph pairs, which make it possible for the first time quickly to generate, store, and evaluate vast quantities of data (Schooley, Hickman & Lane, 1982).

However, a computer will recognize and analyze only what the taxonomist tells it to

recognize and analyze, and new characters are introduced into systematics only through developing new ways of observing and assessing morphology (Hickman, 1977, 1980). In this paper I show how analysis of tooth-row functional integration in the rhipidoglossan radula has led to recognition of sets of characters that are extremely useful in taxonomy. They are characters that, once they have been pointed out, seem so fundamental that it is surprising that they could have been overlooked for so many years by taxonomists.

Functional integration of movement in the radulae of rhipidoglossate marine archaeogastropods involves complex combinations of between-row and within-row interactions of tooth bases and cusps and within-row interactions of tooth shafts. It may also involve interaction facilitated by highly modified plates that no longer function as teeth. Categories of interactions are consistently associated with unique variations on basic morphological themes at higher taxonomic levels. One of the most remarkable features of this integration is revealed in the observation that cusp rows and base rows in the gastropod radula do not necessarily correspond. Integration may, in fact, be so complex as to make it difficult to define rows at all within radulae. These problems and their taxonomic implications are explored below.

METHODS

Uniting taxa on the basis of common functional characters makes one major demand: the taxonomist must be able to infer which aspects of morphology are conservative and to demonstrate that convergence has not occurred. In a previous paper (Hickman, 1980) I identify seven factors that contribute to form and pattern in gastropod radulae and show that underlying phylogenetic factors (conserved groundplans) are readily identifiable. Even where two morphological solutions to the same functional problem are extremely similar, the underlying phylogenetic differences are generally not obscured.

Asymmetry provides an example of a functional character that is very useful in systematics and one that has been overlooked by taxonomists (Hickman, 1981). Asymmetry has developed in radulae with large, strongly-cusped pairs of major food-preparing teeth; it functions to facilitate efficient, alternate folding of these large teeth as the radula is withdrawn from the substrate and into the buccal cavity. It also allows economical, compact storage of teeth in alternate, zipper-fashion within the radula sac. Asymmetry has developed convergently at a superficial level; but when one begins to examine the nature of the asymmetry in detail, one rapidly discovers that it is not simply a presence-absence character. It exists in many different forms or states, and these states are consistently associated in archaeogastropods at the superfamily level (Hickman, 1981).

In the examples that are developed below, morphological and functional data have been obtained from a combination of observations of gastropods radulating glass surfaces and frame-by-frame analysis of slow-motion cinematograpy (Morris & Hickman, 1981); dissections of radulae and manipulations of excised radulae; flat mounts examined with light microscopy; and a combination of single scanning electron micrographs from a variety of angles and stereo paired micrographs (Hickman, 1977).

MORPHOLOGY AND FUNCTION OF THE ROW IN GASTROPOD RADULAE

Descriptions of the molluscan radula consistently refer to the orderly arrangement of teeth in "rows" (e.g. Fretter & Graham, 1962: 169; Hyman, 1967: 236; Purchon, 1968: 45; Solem, 1974: 139; Yonge & Thompson, 1976: 49). The term "transverse row" has been applied by some authors to refer to the full complement of different kinds of teeth (e.g. rachidian, laterals, marginals) that are secreted multiple times during ontogeny from a basic set of genetic instructions. Because each transverse row is repeated numerous times, there are also orderly longitudinal series of teeth. The term "longitudinal row" is confusing, however, and I prefer to refer to teeth in longitudinal series as "columns" (Hickman, 1980). Fig. 1A illustrates a very clear example of orderly arrangement of rows and columns in the radula of a siphonariid limpet, where teeth are relatively undifferentiated. Fig. 1B illustrates rows and columns in a more complex radula in which there is a great deal of differentiation and many different column morphologies.

The distinction between rows and columns is fundamental and obvious and requires no

further discussion. However, having defined a tooth-row as the basic unit of radular morphology, I want to expose a heretofore unappreciated, but serious, discrepancy between *morphological* rows and *functional* rows of teeth.

There is no problem defining a morphological tooth-row if one simply isolates all of the different kinds of teeth within the fundamental unit. These have been illustrated by taxonomists in drawings for more than a century and represented by means of general radular formulae that specify numbers (and sometimes relative sizes and numbers of cusps) of different kinds of teeth.

The difficulty arises in beginning to look at radular function and the ways that teeth interact with one another: at this level we can distinguish "cusp rows" and "base rows." In most radulae cusp rows and base rows coincide; but in others there are remarkable patterns of deviation such that a transverse row of interlocked tooth bases gives rise to cusps that occupy and function in two or three different cusp rows. In some radulae the interactions between teeth are so complex that it is difficult, if not impossible, to define rows in non-arbitrary fashion, even though columns are clearly distinguishable. Deviations from the coincidence of base rows and cusp rows follow patterns that are consistently repeated within higher taxa. Fig. 2 illustrates two func-

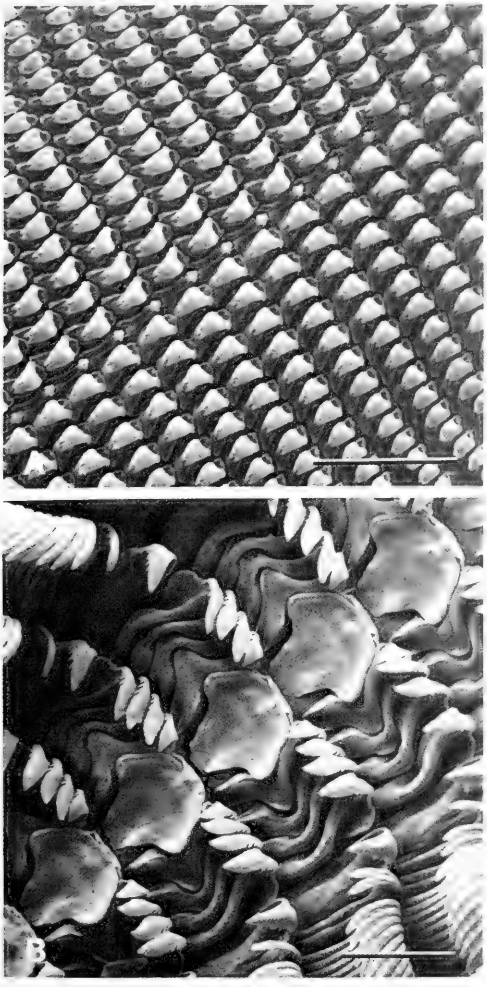

FIG. 1. Radulae in which rows and columns are easily distinguished. A. *Siphonaria* sp., a pulmonate limpet, with a pavement of relatively undifferentiated teeth arranged in rows (diagonals from top left to bottom right) and columns (diagonals from top right to bottom left). Scale bar = 100 μm. B. *Cantrainea panamense* (Dall, 1908), a turbinid with greater differentiation of teeth but retaining distinctive pattern of rows and columns. Scale bar = 40 μm.

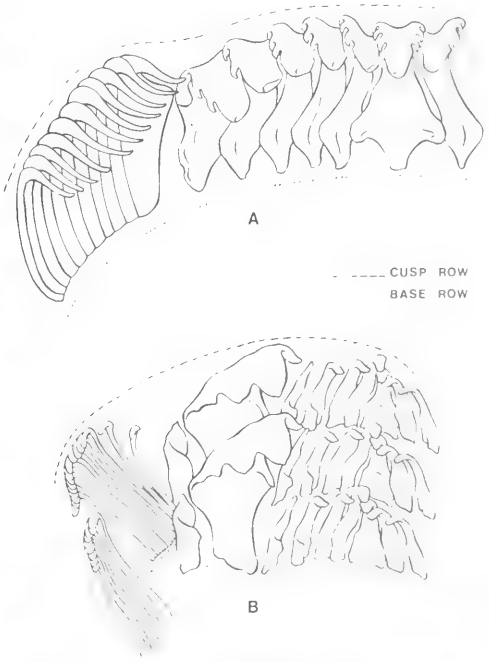

FIG. 2. Two distinct row types in rhipidoglossan radulae. A. The standard condition, exemplified by *Trochus intextus* Kiener, 1850, in which base rows and cusp rows coincide. B. The fissurelline condition, exemplified by *Fissurella nimbosa* (Linné, 1758), the type of the genus, in which cusp rows (dashed line) are distinct from base rows (dotted line).

tionally and taxonomically distinct kinds of rows in rhipidoglossan archaeogastropod radulae.

The standard condition

In Fig. 2A the fundamental pattern of coincidence is illustrated by the radula of *Trochus intextus* Kiener, 1850. In the genus *Trochus* Linné, 1758, and other taxa within the subfamily Trochinae, the bases of the rachidian and lateral teeth are interlocking, with processes that fit in ball-and-socket fashion into depressions on adjacent tooth bases. Between the marginal and lateral teeth there is a specialized marginal tooth with a greatly enlarged and modified base and shaft that serves the function of a lateromarginal plate, facilitating interactions between the two major portions of the row (Hickman, 1980). The most important thing to note is that the cusps in the radula of *Trochus* also overlap and interact within each row and that interacting cusps correspond with interacting bases across each row (Fig. 2A).

In other trochid genera and subfamilies cusps and bases may interact in different ways. For example, in the genus *Gaza* Watson, 1879, lateral tooth bases do not interlock but broadly overlap one another (Fig. 3A), while in the genus *Solariella* Wood, 1842, the pattern of interaction is much more complicated and involves teeth of more highly differentiated morphology (Fig. 3B). But both genera resemble *Trochus* in that tooth shafts are relatively short and give rise to an arcuate row of interacting cusps that correspond to the row of tooth bases. Likewise, in the family Turbinidae, cusp and base rows correspond and basal interactions involve both overlap and interlock (Fig. 3C).

It is reasonable to ask at this point whether cusps, which appear to interact in an excised, flat-mounted radula, actually comprise a functional unit in the living gastropod during feeding. The answer is partially apparent in Figs. 4A and B, where the radula of the northeastern Pacific black turban snail, *Tegula funebralis* (A. Adams, 1854), is illustrated as seen during the feeding stroke. Morris (1980) and Morris & Hickman (1981) have shown that the tooth-rows assume a tight semicircular configuration during feeding due to certain mechanical properties of flexible slit cylinders. Rows maintain their integrity throughout the complicated deformation of the slit cylinder during each feeding stroke. However, microcinematographic frame-by-frame analysis of a feeding stroke demonstrates that the sequence of teeth that passes over a fixed point on the substrate corresponds neither to a sequence of teeth within a row nor to a

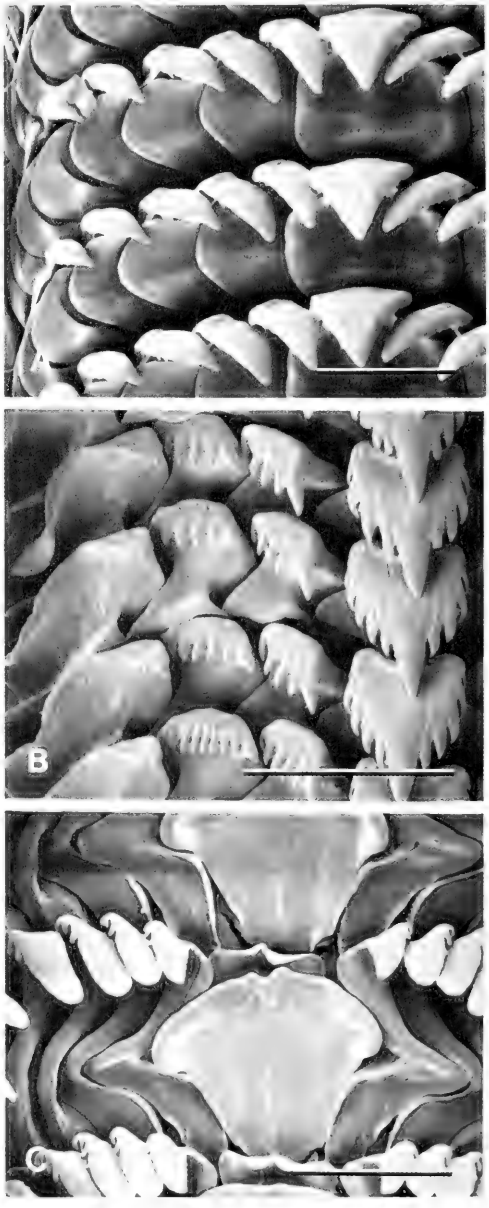

FIG. 3. Basal interactions in trochacean radulae. A. Simple overlap of broadly expanded bases in *Gaza superba* (Dall, 1881). Scale bar = 100 μm. B. Shallow interlock in *Microgaza* sp. Scale bar = 100 μm. C. Deep interlock in *Homalopoma carpenteri* (Pilsbry, 1888). Scale bar = 40 μm.

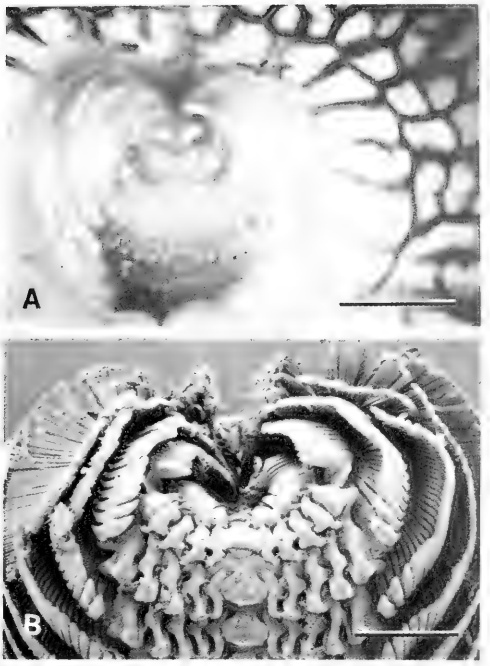

FIG. 4. Operational configuration of tooth-rows in the radula of *Tegula funebralis* (A. Adams, 1854). A. Optical micrograph of feeding stroke of a living snail. Scale bar = 1 mm. B. Scanning electron micrograph of an artificial protrusion of radula into same functional conformation. Scale bar = 400 μm. Note striking difference between tight semicircular configuration of functioning row and standard configuration illustrated in flat radula mounts (from Morris & Hickman, 1981).

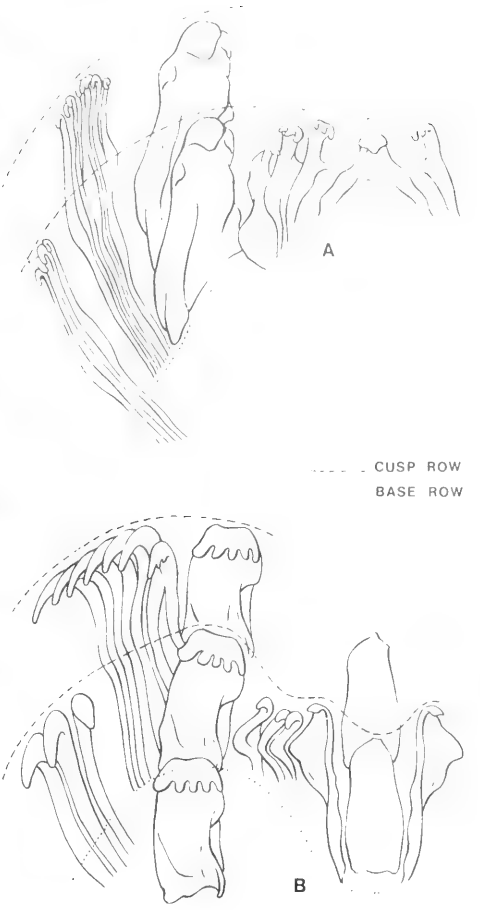

FIG. 5. Alternative configurations of base rows and cusp rows in the cocculinacean radula. A. Typical pattern in the genus *Cocculina* Dall, 1882, in which an arcuate base row (dotted line) gives rise to long-shafted marginal teeth that function in a different cusp row from the massive outer lateral tooth. B. Typical pattern in the genus *Pseudococculina* Schepman, 1908, showing same distinctive pattern of marginal shaft elongation associated with teeth of different morphology and base row configuration.

sequence within a column (Morris, 1980; Morris & Hickman, in prep.). It is, nevertheless, an elegant sequence of successively refined morphologies for efficiently gathering food particles.

The cocculinid condition

The row becomes more difficult to define in small rhipidoglossan limpets of the deep-sea, wood-ingesting genus *Cocculina* Dall, 1882. Tooth cusps are arranged in orderly arcuate rows, but careful attempts to follow the cusps in any one row back to their bases demonstrates that marginal cusps arise from bases in a different row from the lateral cusps.

Figs. 5A, 6A and C illustrate the condition in *Cocculina*. It results from a pronounced elongation of marginal tooth shafts. The shafts are laterally flattened, thin, and expanded (Fig. 6C) in contrast to the semicircular or quadrate cross-sectional shape of the typical rhipidoglossan marginal tooth. The terminal cusp is relatively small and finely serrate. Shafts do not have expanded bases and arise from the radular membrane adjacent to the bases of a very large, robust, and heavily reinforced outer lateral major food-preparing tooth. Shafts extend well beyond (anterior to) the cusp of the large outer lateral, however, terminating in a row of small cusps that lie alongside the outer lateral cusp two rows anterior (Fig. 6A).

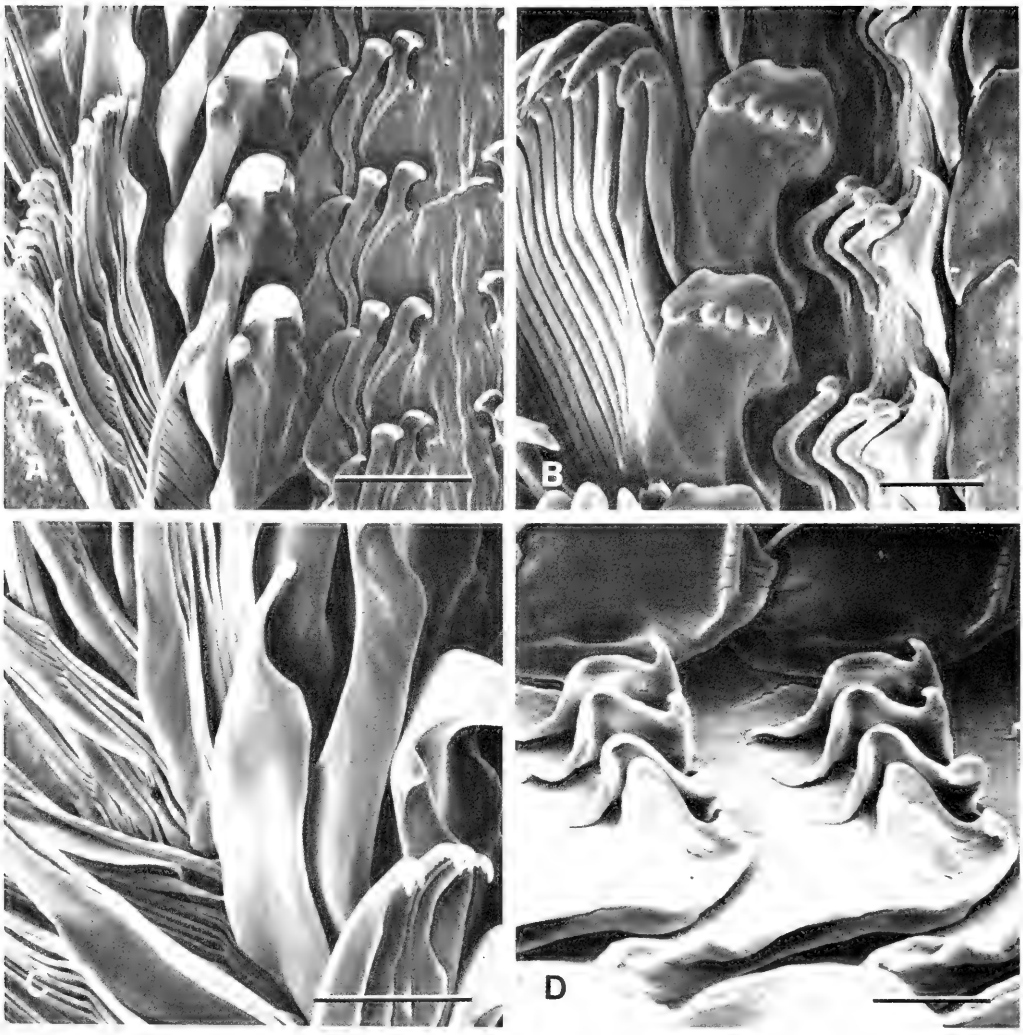

FIG. 6. Basic features of the cocculinacean radula. A. Row configuration and tooth morphologies in *Cocculina* sp. Scale bar = 40 μm. B. Row configuration and tooth morphologies in *Pseudococculina* sp. Scale bar = 20 μm. C. Detail of flattened marginal tooth shaft and small hooked cusp in a second species of *Cocculina*. Scale bar = 40 μm. D. Low-angle detail of the oval rachidian and interlock system of left lateral teeth in a second species of *Pseudococculina*. Scale bar = 20 μm.

A strikingly similar discrepancy between base row and cusp row appears in radulae in the deep-sea limpet genus *Pseudococculina* Schepman, 1908 (Figs. 6B and D). This is interesting because teeth in the radula of *Pseudococculina* are of such a different form as to suggest different familial status. The rachidian teeth are well developed and relatively thick, oval, overlapping plates; and the inner laterals are better developed and interlock basally when the radula is collapsed (Fig. 6D). The innermost lateral has an unusually long inner basal leg, and the tooth is attached basally along a broad diagonal area extending from the posterior end of the rachidian to a position that is anterior to the anterior end of the rachidian. The outer lateral tooth is also greatly enlarged, but it has a very different pattern of cusps and a depression on its inner face to accommodate the basal leg of the adjacent lateral tooth. The marginal teeth are more nearly circular in cross section and the shafts terminate in large, hooked cusps. However, they are extraordinarily long, as in

Cocculina, and they arise from a lateral base row posterior to the rows into which their cusps hang and interact (Fig. 5B).

Elongation of marginal tooth shafts has not been observed to date in other rhipidoglossan archaeogastropods and constitutes a powerful argument for considering relatively close phylogenetic affinities between the two genera. The Cocculinacea is currently a garbage-basket taxon for a diversity of minute deep-sea limpets of diverse radular morphology, some lacking marginal teeth. Analysis of row interactions may provide more powerful clues to intrageneric phylogenetic relationships than details of comparative tooth morphology.

The fissurelline condition

A more complicated form of discrepancy between base rows and cusp rows appears consistently in radulae of keyhole limpets of the genus *Fissurella* Bruguière, 1789 (Figs. 2B and 7). The radula is strikingly asymmetric (Hickman, 1981), to accommodate storage of enlarged, heavily-cusped, outer lateral food-preparing teeth. The rachidian is asymmetric, has a narrow simple cusp, and is flanked by four simply cusped inner lateral teeth. The enlarged, massive, outer lateral is twice the height of the rachidian and inner laterals, so that, while its base is aligned with the bases of one set of lateral teeth, its cusp is aligned with the cusps of the next anterior row. A striking feature of the fissurelline radula is the well-developed, uncusped, lateromarginal plate that lies between the large outer lateral and the marginal teeth as a specialized articulatory structure (Hickman, 1976, 1977, 1980). The posterior margin of the plate is aligned with the posterior margins of the rachidian and laterals, and thus it is not difficult to assign to a base row. The innermost marginal teeth, however, do not arise adjacent to the lateromarginal plate, but rather from behind or slightly anterior to the plate (see Fig. 2B and the isolated plate and inner marginals in Fig. 7B).

From a functional or biomechanical point of view, each lateromarginal plate interacts with two rows of marginal teeth (Hickman, 1980). The shallow pocket on the back of the plate fits over the lower portion of the shafts that arise behind it, so that the plate can assist in pushing those marginals out into feeding position. A similar pocket accommodates the mid-portion of the shafts of the next posterior

FIG. 7. Basic features of the fissurelline radula. A. Row configuration and tooth morphologies in *Fissurella nimbosa* (Linné, 1758). Scale bar = 400 μm. B. Detail of isolated lateromarginal plate from *F. volcano* Reeve, 1849, and posterior set of marginals with which plate interacts. Scale bar = 100 μm.

row of marginals and assists in their collapse and in maintaining their alignment. Thus each row of marginal teeth also interacts with two adjacent lateromarginal plates. The shafts of the marginal teeth are so long (three times the length of the rachidian and inner laterals) that they function in a different cusp row from both the rachidian and inner laterals on one hand and the massive outer lateral on the other (Fig. 2B).

The lateromarginal plates also have connections along the length of the column to one

another, so that forces will be transmitted sequentially along the column during the feeding stroke. This situation is sufficiently complicated that it is difficult, if not impossible, to define a row in a functional sense. It is interesting in this respect that, in the early part of the century, Torr (1914), using light microscopy, observed that more than one set of marginal teeth seemed to be associated with each of the elongate rhomboidal plates. In his illustrations he shows a single tooth row as consisting of two ranks of marginal teeth attached at their bases along one side of the plate. It is clear from his text descriptions that he believed there had been a duplication of marginals, although this is clearly not the case.

The complicated situation described above with respect to interactions between marginal teeth and lateromarginal plates is characteristic not only of the Fissurellinae, but occurs in the other fissurellid subfamilies as well. The lateromarginal plate is not so highly developed in the other subfamilies, however. The taxonomic implications of lateromarginal plate functional morphology are discussed further in a following section. On the other hand, the discrepancy between cusp and base rows created by enlargement of the outer lateral tooth does seem to be restricted to the Fissurellinae. In other fissurellid subfamilies the outer lateral tooth, although massive, has not doubled in length; and its base and cusp thus correspond with bases and cusps of the adjacent lateral teeth (Fig. 8).

Inferences of an advanced, complicated, and highly derived state of functional interactions within the fissurelline radula parallel evidence from the geologic record and strengthen phylogenetic inferences. The fissurelline shell does not appear until the Eocene, while emarginuline and diodorine species appear early in the Mesozoic (Triassic and Jurassic) (Knight *et al.,* 1960).

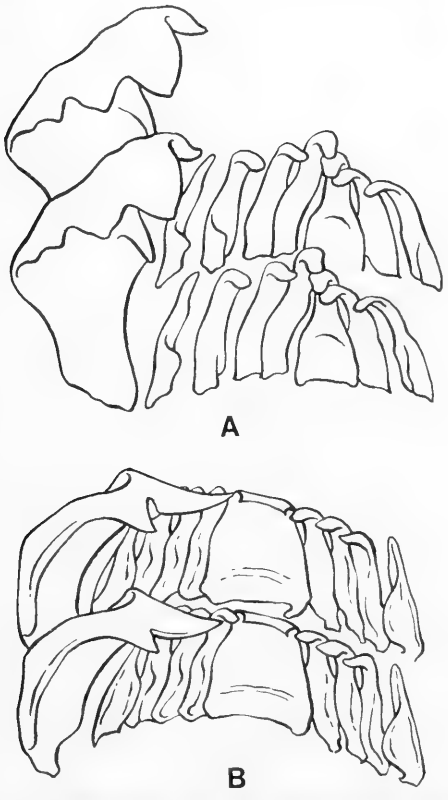

FIG. 8. Alternative cusp row configurations in two fissurellid subfamilies. A. Subfamily Fissurellinae: the massive outer lateral tooth with base and cusp situated in different rows. B. Subfamily Diodorinae: the massive outer lateral tooth with its base and cusp situated in the same row.

The neritacean condition

A final example of a unique pattern of toothrows is seen in neritacean gastropods. The

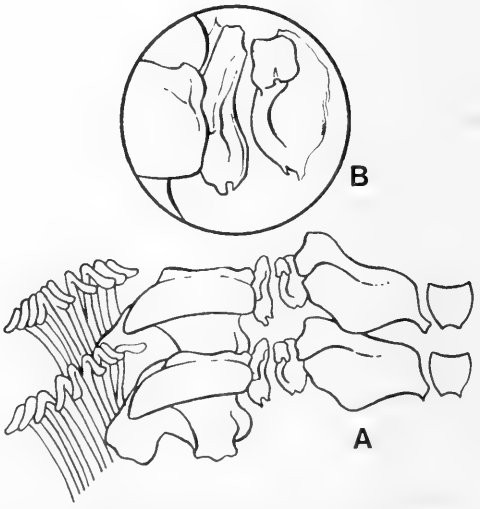

FIG. 9. The neritacean condition. A. Row configuration and basic tooth morphologies based on radula of *Nerita plicata* Linné, 1758. Note how the flange on the fouth lateral tooth fits between and interacts with two sets of marginal teeth. B. Detail of the simultaneous interaction of the third lateral tooth with the base (anterior) of one fourth lateral and the cusp (posterior) of another (see also Fig. 11).

complications are also related to functional interactions of tooth bases and cusps. Some important features of radulae from two neritacean families, the Neritidae and Phenacolepadidae, are illustrated in Figs. 9 and 10.

There is a broad central region in the typical neritacean radula that is occupied by rows of "teeth" that are modified into complex plates of a variety of shapes. Baker (1924) has described the basic forms of these plates in some detail. The terms "base," "shaft," and "cusp" are not readily applicable. They are better described in terms of notches and processes. Briefly, the small quadrate rachidian is flanked by a broad first lateral and relatively small, but thick and complexly curved, plate-like second and third laterals. There is no problem defining rows in this central region of the radula. A major hallmark of the neritacean radula is the massive, compound, fourth later-

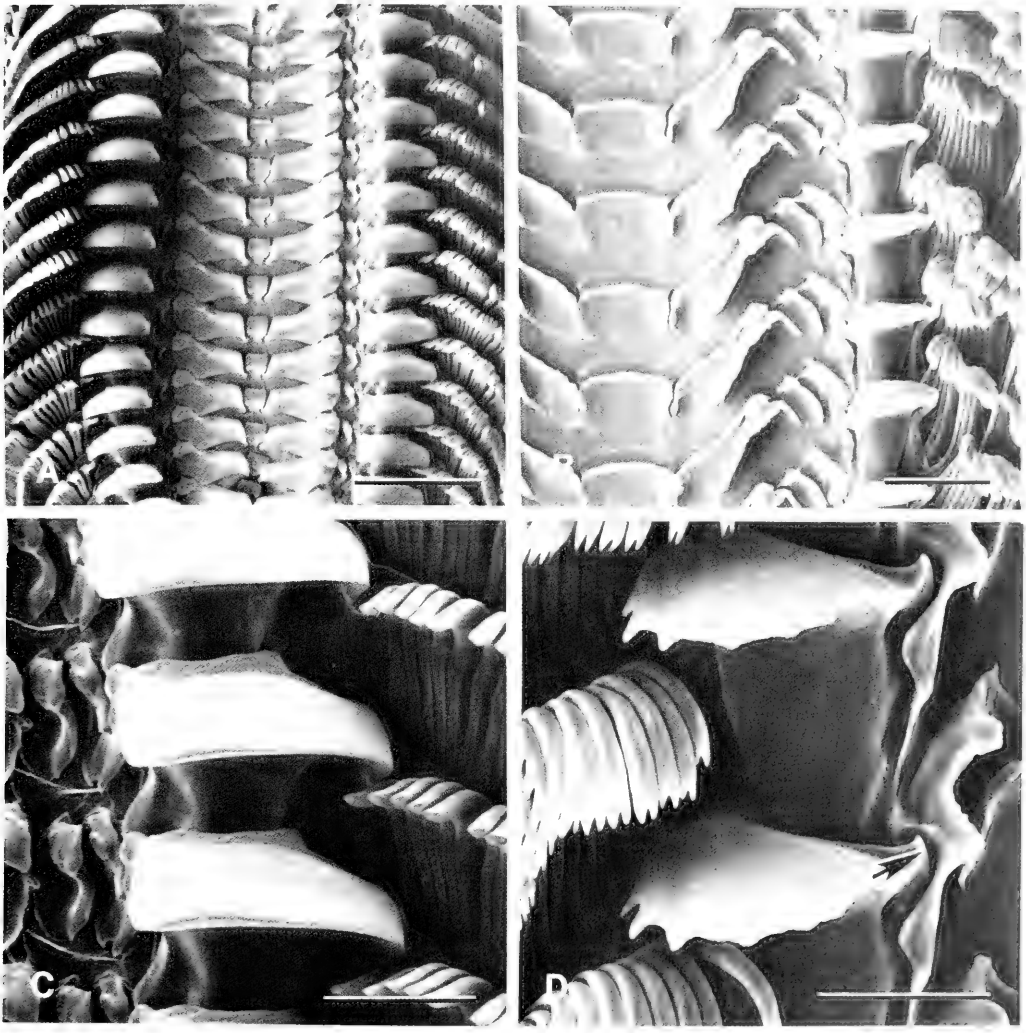

FIG. 10. Basic features of the neritacean radula. A. *Nerita undata* Linné, 1758: row configuration and tooth morphologies. Scale bar = 400 μm. B. *Phenacolepas osculans* (C. B. Adams, 1852), a neritacean limpet: row configuration and tooth morphologies. Scale bar = 40 μm. C. *Nerita plicata* Linné, 1758; detail of interaction of the massive fourth lateral with inner laterals (left) and marginals (right). Note also the alternation of cusp edges of marginals with cusps of fourth laterals. Scale bar = 100 μm. D. *Phenacolepas* sp.: detail of interlock of fourth lateral cusp and third lateral (at arrow). Compare alternation of marginal cusp edges and fourth lateral cusps with Fig. 10C. Scale bar = 20 μm.

al tooth with its heavy base, broad, spoon-shaped cusp, and lateral flange. The placement of the base and cusp make it difficult to decide to which inner plate row it belongs: the base interacts with the anterior end of one of the small third laterals while the large spoon-shaped cusp interacts with the posterior end of the small third lateral in the next anterior plate row. In other words, we are looking at an alternation of cusp-base, base-cusp interactions. The ambiguity of the situation can be appreciated by examining the inset in Fig. 9. Baker's (1924) drawings of neritid radulae imply one interpretation, while Fretter's (1965) drawing implies the alternative; and it probably makes little difference how one chooses to define a row in this instance. From some vantage points and with the radula in certain configurations, one set of interactions may appear more important than another: the interaction of the third lateral plate with the fourth lateral cusp is emphasized in the preparation and viewing angle of Fig. 11. The same kind of intimate connection of the third lateral simultaneously to two fourth laterals can be seen in the Phenacolepadidae (Fig. 10B).

There is also a unique neritacean pattern of interactions between the massive outer lateral tooth and the marginal tooth-rows. Looking at the unfolded or flattened radula of *Nerita* Linné, 1758, or *Phenacolepas* Pilsbry, 1891 (Figs. 10C and D), there is a distinct alternation or interleaving along the column axis of the broad cutting edges of the outer laterals and the compound edges presented by the cusps of the marginal teeth. This interleaving is interpreted as a functional necessity for efficient tooth storage: an alternative to the pronounced asymmetry that has developed in many other rhipidoglossan groups to help deal with accommodation of enlarged major substrate-excavating teeth (Hickman, 1981). Again, there is a question as to which marginal teeth belong with which outer laterals; and, again, there is no clear-cut answer.

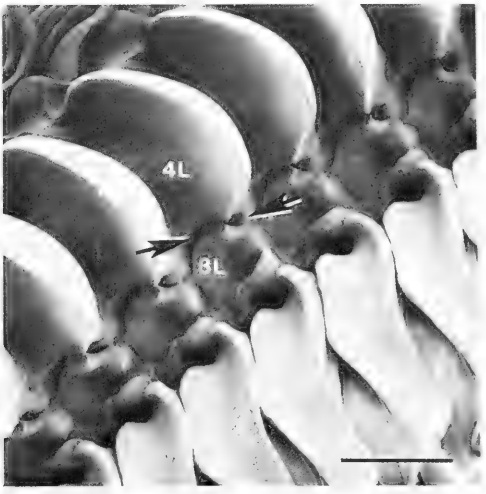

FIG. 11. *Nerita funiculata* Menke, 1851: tilted view of a neritacean radula emphasizing interaction of third lateral (3L) with base of one fourth lateral (anterior arrow) and the cusp/shaft (posterior arrow) of another. Scale bar = 100 μm.

From the foregoing discussion of the row concept in the gastropod radula, it can be concluded that there is only one kind of row in a strictly morphological sense and that all gastropod radulae have a fixed set of morphological units that is repeated numerous times. However, there are other kinds of rows in a functional sense. Base rows and cusp rows may not coincide, and major patterns by which they are defined and functionally interactive are useful as characters in taxonomy and in drawing phylogenetic inferences. In the most complicated kinds of functional integration it is probably not useful to worry about defining rows, but rather to focus on understanding and characterizing the interactions of elements within the radula. In the following sections, I provide some examples of specific interactions that are of interest from a systematic point of view.

CUSP INTERACTIONS

In the preceding section, cusp rows were distinguished as transverse series of food-preparing and food-gathering units that are situated adjacent to one another regardless of whether their bases are adjacent and interacting. Cusps interact with one another in a variety of ways during feeding and during storage of the radula when the animal is not feeding.

Many cusp interactions are not reflected in morphology; but, particularly in the major substrate-excavating teeth, cusps may display adaptations from transmitting forces to one another. Although these adaptations may look superficially very similar (convergence), different groups of gastropods have developed different ways of solving similar problems.

For example, in both the genus *Haliotis* Linné, 1758, and the genus *Turbo* Linné, 1758, there has been reduction in the prominence of the rachidian and lateral teeth and enlargement of the inner marginal tooth cusps, with accompanying development of accommodational asymmetry to the tooth-rows (although note that the rows slope in opposite directions) (Hickman, 1981). The basic patterns are illustrated for comparison in Figs. 12A and B. The outer marginal cusps in both genera overlap and interact without any physical modification of cusps. But in both genera the cusps of the three inner marginal teeth are modified physically to interlock (Figs. 12C and D). In *Turbo* note at the arrow in Fig. 12D how the secondary cusp is accommodated by a strong depression on the top of the main cusp on the adjacent tooth. In *Haliotis* note at the arrow in Fig. 12C that the

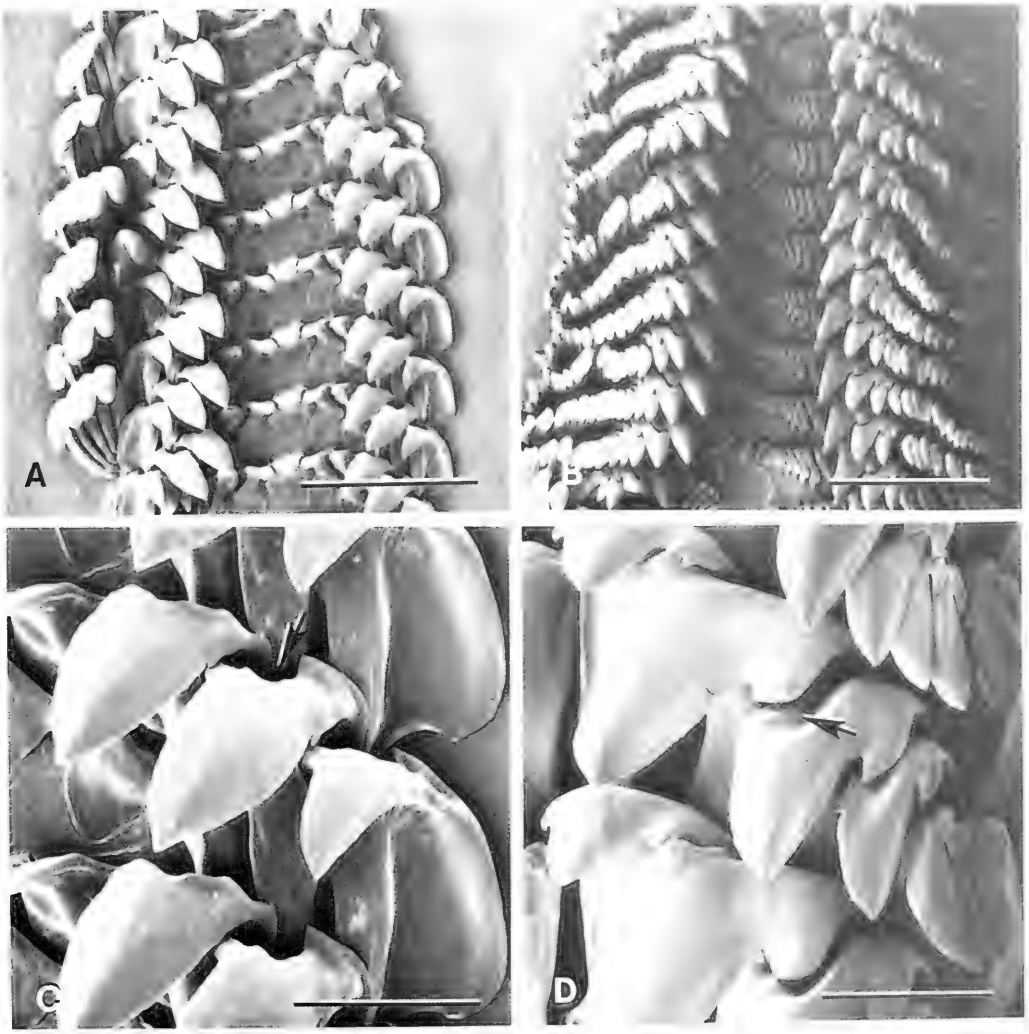

FIG. 12. Asymmetric radular patterns and convergent cusp interaction in the Haliotidae and Turbinidae. A. *Haliotis rufescens* Swainson, 1822. Scale bar = 400 μm. B. *Turbo fluctuosus* Wood, 1828. Scale bar 500 μm. Note that both radulae are asymmetric but that the direction of slope is reversed. C. Cusp interactions in *H. rufescens*: note at arrow how the edge of one cusp fits into notch at the back of the adjacent cusp. Scale bar = 100 μm. D. Cusp interactions in *T. fluctuosus*: note at arrow how secondary cusp fits into depression on top of the adjacent cusp. Scale bar = 100 μm.

main cusps overlap in the same manner that they do in *Turbo* but that the back of the cusp fits into a notch at the rear of the cusp on the adjacent tooth and that the adjacent tooth cusp bears a process that fits into a pocket beneath the neighboring cusp.

Although the fine, serrate, outer-marginal teeth that are so characteristic of the rhipidoglossan radula generally do not develop pronounced physical accommodations to one another, there are some interesting instances of marginal tooth interactions. For example, Fig. 13 shows how a long process has developed near the base of the serrate margin of the cusp in an undescribed rhipidoglossan deep-sea limpet. It wraps around and under the adjacent cusp, limiting the degrees of freedom of movement. A series of marginal teeth can thereby become linked and aligned as a unit.

Standard methods for preparing radulae generally destroy evidence of the ways that teeth are aligned during feeding. This is particularly true of the delicate marginal teeth, which must be folded into an unnatural configuration when an excised radula is mounted flat for either optical or scanning electron microscopic viewing. Morris (1980) has developed a method for producing artificial protrusions of gastropod radulae that preserve details of cusp relationships and interaction as they exist during feeding (Morris & Hickman, 1981). Marginal tooth cusps contact the substrate as a unit. Fig. 14 shows how in *Tegula funebralis*, marginal tooth cusps in the tight semicircular configuration of the radula during the feeding stroke overlap one another to form a long, continuous, serrate, food-collecting edge along the length of the row.

SHAFT INTERACTIONS

Interactions of tooth shafts also provide an important means of integrating function and transmitting forces. There is virtually no systematic documentation in the literature of patterns of variation in shaft length, cross-sectional shape, and flexural stiffness in radular teeth. Even less is understood about the biomechanical or functional significance of interactions of length, shape, and stiffness. Rachidian and lateral tooth shafts are generally shorter, reinforced into more complex cross-sectional shapes, and less flexible than the shafts of marginal teeth. It makes intuitive sense that the substrate-preparing teeth should have a different set of properties from the brushing teeth that gather up food particles.

One of the most interesting, recurring adaptations in rachidian and lateral teeth is development of thin, laterally-extended flanges of chitin off the backs of tooth shafts. These are frequently joined with tooth cusps and are interleaved in complex ways with similar shaft extensions of adjacent teeth.

FIG. 13. Marginal tooth cusp interactions in an undescribed deep-sea limpet of uncertain affinities from the Galápagos Rift. Note at arrows how enlarged processes wrap around cusps of adjacent teeth. Scale bar = 10 μm.

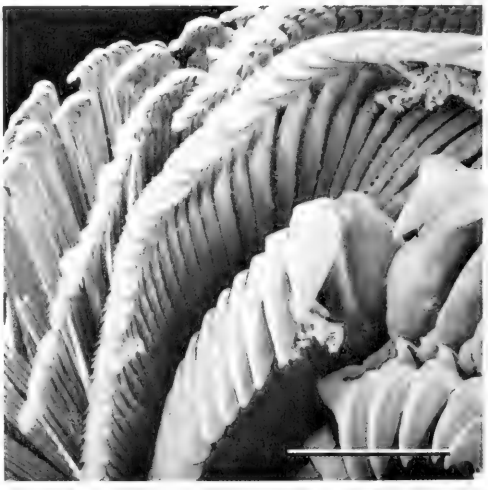

FIG. 14. *Tegula funebralis*: artificial protrusion of radula in operational configuration showing how cusps of marginal teeth overlap to form a continuous food-collecting edge. Scale bar = 200 μm.

These shaft modifications are important from a systematic point of view because different distinctive patterns have evolved at higher taxonomic levels and characterize major groups.

For example, a complex of closely-related genera of deep-sea deposit-feeding trochid gastropods (*Bathybembix* Crosse, 1893; *Cidarina* Dall, 1909; *Calliotropis* Seguenza, 1903) has developed a particularly elaborate set of expanded or "hooded" rachidian and lateral teeth that must greatly alter the magnitudes and distributions of stress that would occur in free-standing teeth with simple tooth shafts. Fig. 15A illustrates the pattern of interleaving of teeth that results. A somewhat simpler form of shaft expansion and interaction occurs in closely-related trochaceans of the genus *Turcica* A. Adams, 1854 (Fig. 15B).

FIG. 15. Shaft interactions in two trochid gastropods. A. *Calliotropis regalis* (Verrill & Smith, 1880): rachidian and lateral teeth with broad interleaving shaft expansion or "hoods." Scale bar = 400 μm. B. *Turcica caffea* (Gabb, 1865): shaft expansion and interleaving of a similar type. Scale bar = 40 μm.

Shaft expansion occurs prominently and consistently at one critical point in the radula of neritacean gastropods: between the massive fourth lateral and the marginal tooth complex. There is a well-developed thin extension of the shaft that is joined to the cusp and passes laterally behind the shafts and cusps of the inner marginal teeth of one row, separating them from the shafts of the next anterior marginal tooth-row (Figs. 9, 10C). Expansions of this sort frequently stain well with protein-specific stains, suggesting tanning of the chitin into a more rigid structure that would function effectively in transmitting stress without excessive deformation.

Interactions between marginal tooth shafts do exist, but they are of a different nature from rachidian and lateral tooth interactions. They involve primarily fusion of adjacent shafts or, alternatively, incomplete separation of tooth shafts during radular ontogeny. Particularly in radulae in which the marginal teeth are numerous and of very thin, light construction, the lowermost portions of the shafts will be fused. The fusion in such cases is generally restricted to a region below a pronounced twist in the shafts and where the shafts come to lie parallel to the radular membrane (Fig. 16).

FIG. 16. Shaft fusion in *Trochus intextus*. Note at arrows that outer marginal tooth shafts are united. Scale bar = 100 μm.

Incomplete separation of marginal teeth is relatively common in rhipidoglossate radulae, and it is not clear to what extent the condition may be adaptive and to what extent it may represent a relaxation of selection for separation, tanning, and hardening of each tooth individually (Hickman, 1980). Incomplete separation of teeth seems to be more frequent in deep-sea gastropods. Fig. 17A illustrates fusion of both marginal tooth shafts and cusps in the Galápagos rift limpet, *Neomphalus* McLean, 1981. Fig. 17B, on the other hand, illustrates what is better termed bifurcation or multifurcation, in which a single shaft clearly gives rise, in more or less orderly pattern, to two or more cusps. The animal in this instance is an undescribed trochacean from hydrothermal vents at 21°N on the East Pacific Rise.

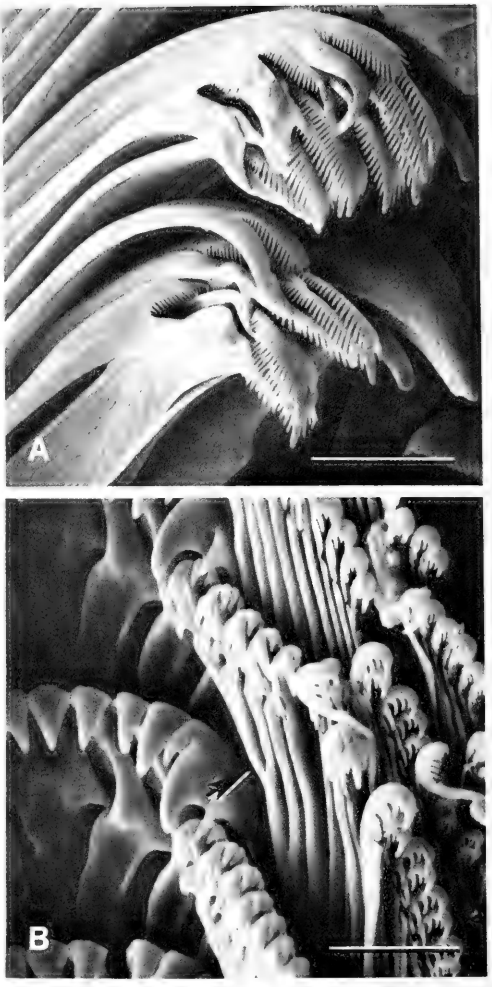

FIG. 17. Joining of marginal tooth shafts. A. *Neomphalus fretterae* McLean, 1981: marginal tooth shafts and cusps showing what is interpreted as irregular and partial separation. Scale bar = 20 μm. B. Undescribed deep-sea trochacean microgastropod with branching marginal tooth shafts (at arrows). Scale bar = 10 μm.

BASAL INTERACTIONS

Patterns of basal interaction between radular teeth can be particularly useful in molluscan systematics. Basal expansion has led to many unique methods of overlapping and interlocking, both within rows and along columns, that are conserved at higher taxonomic levels.

I pointed out previously (Hickman, 1980) that basal interactions are important biomechanically because they perform multiple functions. First, they act in dissipating stress applied to the radular membrane by any one tooth. Second, they provide an effective means of dealing with bending and overturning movements on individual teeth. Third, they can assist in aligning and orienting teeth during feeding and in activating one another sequentially during the feeding stroke. And finally, they can assist in folding teeth together efficiently and economically to occupy the least possible space while the radula is not in use. The interactive potential of tooth bases was not well understood until the scanning electron microscope became available, and SEM studies of a diversity of molluscan radulae all suggest that basal interactions are an important functional feature (Solem, 1972, 1973, 1974; Solem & Richardson, 1975; Solem & Roper, 1975; Bertsch *et al.*, 1973; Bertsch & Ferreira, 1974; Ferreira & Bertsch, 1975).

Basal overlap and interlock were used in the foregoing discussion to define base rows, and several illustrations were provided in the context of those earlier remarks (Figs. 3A–C). Many rhipidoglossan families, subfamilies, and genera have developed distinctive forms of basal interaction between rachidian and lateral teeth. They are best developed in taxa that feed on hard substrates in the rocky intertidal and shallow subtidal zones. Deposit-feeding taxa may have interlocking teeth (e.g. Fig. 15A), although the processes and corresponding sockets are of more delicate construction.

A different form of basal interrelationship is associated with filter feeding. Various lines of evidence have led Fretter (1975) and, independently, Hickman, McLean & Ponder (unpublished data) to recognize or suspect filter-feeding in a variety of archaeogastropods. In filter-feeding taxa, tooth shafts and cusps tend to be lost; and bases, likewise, may be reduced to thin chitinous vestiges, as in *Umbonium* Link, 1807, and *Bankivia* Krauss, 1848 (Figs. 18A and B). The situation is less clear in the genus *Lirularia* Dall, 1909. Two of the eastern Pacific species, *L. succincta* (Carpenter, 1864) and *L. lirulata* (Carpenter, 1864), have what I have identified as a filter-feeding radula; however, McLean (personal communication) notes that the gill, although unusual, is not a typical filter-feeding gill. In the western Pacific species *L. iridescens* (Schrenck, 1863), bases form a distinctive pavement of interacting teeth separating the marginal tooth complexes (Figs. 18C & D).

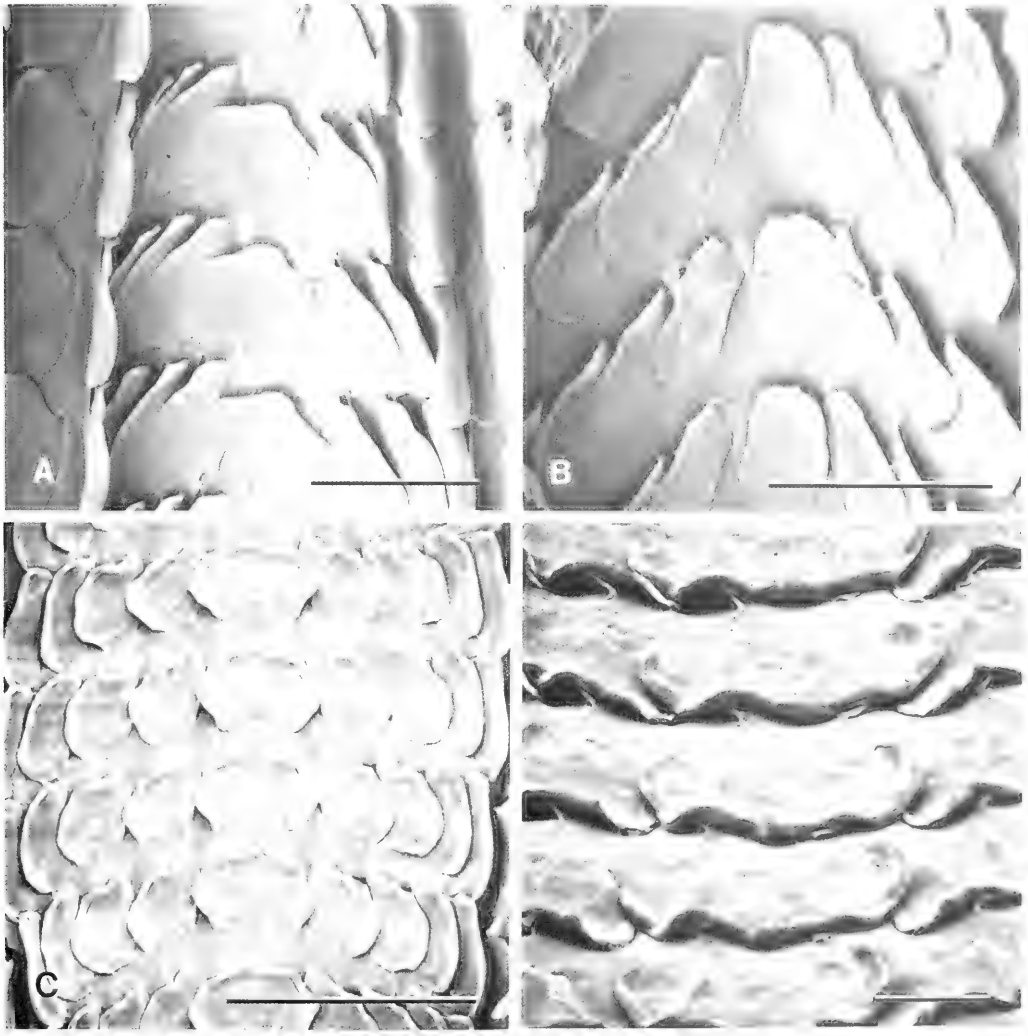

FIG. 18. Degenerate rachidian and lateral teeth of recognized or suspect filter-feeding trochaceans. A. *Umbonium (Ethalia) guamense* (Quoy & Gaimard, 1834): central portion of radula reduced to thin, vestigial tooth bases. Scale bar = 100 μm. B. *Bankivia (Leiopyrga) lineolaris* (Gould, 1861): similar reduction of teeth to vestigial bases. Scale bar = 40 μm. C. *Lirularia iridescens* (Schrenck, 1863): rachidian and laterals lacking cusps and shafts, but not so reduced as in *Umbonium* and *Bankivia*. Scale bar 100 μm. D. *L. iridescens*: low angle view of overlapping pavement of basal plates. Scale bar = 20 μm.

The function of the radula in filter-feeders is not clearly understood, and not all taxa that are able to do so are obligate filter feeders. Although *Neomphalus* can filter-feed, it retains well-developed rachidian and lateral teeth and the capacity to graze (McLean, 1981). In any event, the characteristics of reduced radular teeth should prove helpful in distinguishing the phylogenetic origins of filter feeding in different archaeogastropod taxa.

Evolutionary degeneration of rachidian and lateral teeth, accompanied by reduction in the degree of interaction between tooth bases, has occurred in non-filter-feeding taxa as well and has already been illustrated for the Cocculinacea (Figs. 5 and 6) and Fissurellacea (Figs. 2 and 7). Here it is related to the development of massive outer lateral or inner marginal substrate-preparing teeth that are used on a variety of organic substrates (wood, encrusting sponges, compound ascidians, bryozans, etc.). Highly developed basal interactions in these taxa are associated with the massive food preparing tooth; and, in the Fissurellacea, with the lateromarginal plate.

The more highly developed and refined a basal interaction becomes, the more useful it should be in taxonomy if the refinements are taxon-specific. Lateromarginal plates may be considered a special case of extreme modification of tooth bases; and, because modification has proceeded along very different lines in different rhipidoglossan groups, they provide a whole series of potentially useful characters. Hickman (1976, 1977, 1980) has emphasized the functional importance of lateromarginal plates as articulatory structures between substrate-preparing and food-gathering portions of rhipidoglossan radulae (i.e. between lateral and marginal teeth), and the highly developed condition of these plates in the fissurelline radula was discussed earlier in this paper.

I am currently developing a set of characters and character states for lateromarginal plates that includes: presence/absence of the plate; condition of the plate with respect to shaft and cusp (short shaft and well-developed cusp present; long shaft and well-developed cusp present; long shaft and rudimentary cusp present; shaft and cusp absent) lateromarginal plates linked/not linked to one another; lateromarginal plates modified bases of lateral teeth/modified bases of marginal teeth; plates with/without interaction with outer lateral tooth; plates with/without interaction with inner marginal teeth.

Examples of some character states with respect to shaft and cusp are illustrated in Figs. 19A–D. In the genus *Diloma* Philippi, 1845, there is a very short shaft and well-developed cusp that resembles the cusps of other marginal teeth (Fig. 19A). This state is also characteristic of genera and species within the subfamily Trochinae. In *Solariella* Wood, 1842 (Fig. 19B), and *Calliotropis* Seguenza, 1903 (Fig. 19C), the lateromarginal plate has a long but rudimentary shaft and cusp. In *Fissurella* Bruguière, 1789, the plate is well developed and completely lacks shaft and cusp, although well-developed shafts and cusps are present in other fissurellid subfamilies.

SUMMARY AND CONCLUSIONS

Although functional characters must be used with great care in classification and phylogenetic inference, they can be used to generate new sets of taxonomic characters and can provide important insight into relationships. Functional characters are particularly useful in archaeogastropod systematics when applied to rhipidoglossan radulae. Studies of tooth-row functional integration lead not only to new systematic insights, but they are also helpful in understanding how radulae work in preparing and gathering a diversity of kinds of food from a diversity of substrates. Some of the more important conclusions are:

1. Although interactions between radular teeth occur primarily within rows and within columns, tooth interactions with the substrate may follow a more complex sequence that is related neither to rows nor columns.
2. Although cusp rows and base rows coincide in many rhipidoglossan gastropods, and although there is only one kind of row in a strictly morphological sense, there are many kinds of rows in a functional sense. Base rows and cusp rows frequently do not coincide, and the major patterns by which they are defined and functionally interactive provide useful characters in systematics.
3. In the most complicated kinds of functional integration it is not practically possible to define tooth-rows in a functional sense.
4. Complex patterns of tooth interactions are consistently associated at higher taxonomic levels.
5. Cusp interactions include adaptations for transmitting forces from one tooth to another and frequently involve distinctive modifications of morphology. Even where there

are no strong morphological modifications, many cusps may be presented to the substrate as a functional unit.

6. Shaft interactions also integrate function and transmit forces according to characteristic patterns in different taxa. Shaft expansion and interleaving has been superimposed on a number of different radular groundplans, and fusion of shafts and incomplete separation of shafts can alter both the appearance and biomechanical properties of teeth.

7. Patterns of basal interaction are functionally important because they perform many functions simultaneously. Patterns of overlap and interlock are different in different taxa. Lateromarginal plates are specialized tooth bases that are important as articulatory structures. They have developed between the substrate-preparing and food-gathering portions of many rhipidoglossan radulae. There are both morphological and functional characters associated with these plates that are particularly useful in systematic assessments.

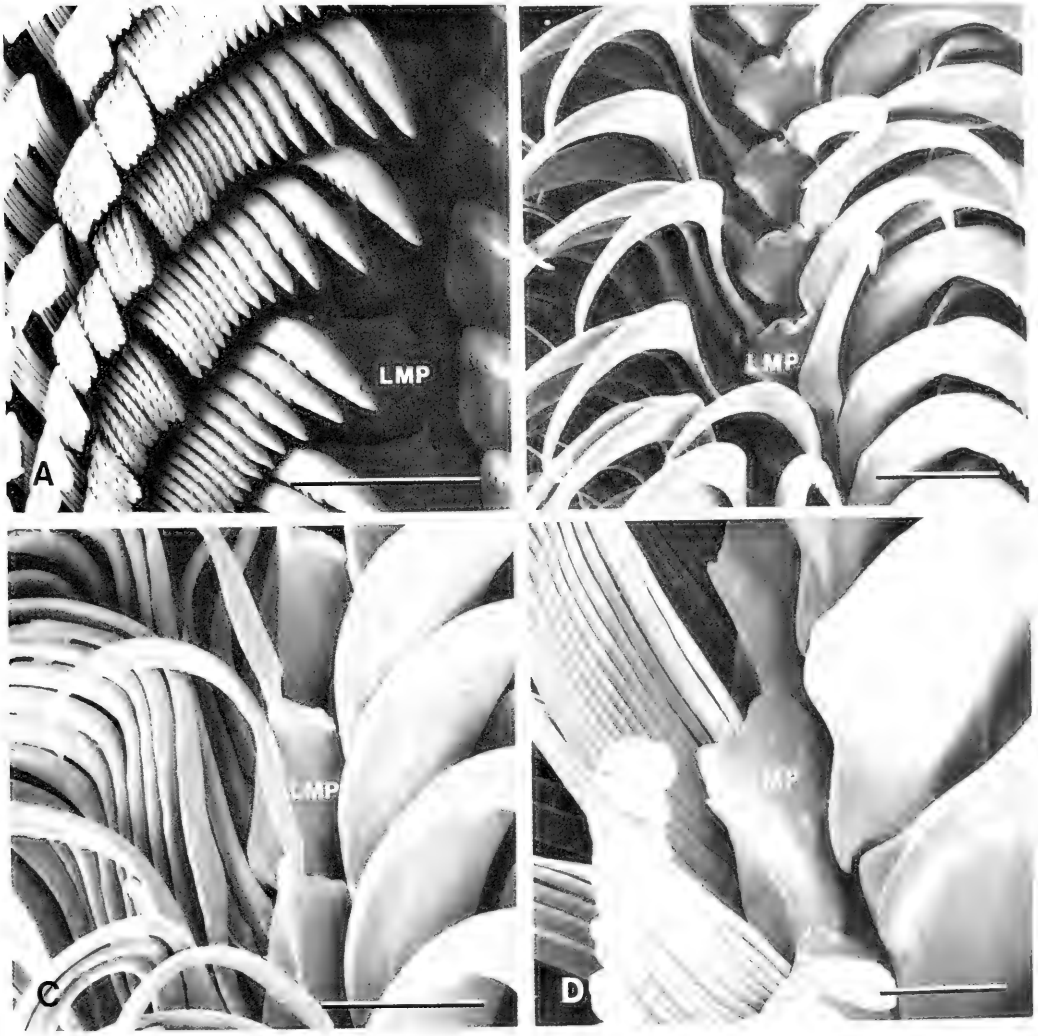

FIG. 19. Examples of lateromarginal plates (LMP). A. *Diloma nigerrima* (Gmelin, 1791): broad plate with well-developed short shaft and cusp. Scale bar = 100 μm. B. *Solariella nuda* Dall, 1896: thin plate with elongate rudimentary shaft and cusp. Scale bar = 100 μm. C. *Calliotropis hataii* Rehder & Ladd, 1973: well-developed interactive plate with long rudimentary shaft and cusp. Scale bar = 50 μm. D. *Fissurella volcano* Reeve, 1849: massive interactive plate lacking shaft and cusp. Scale bar = 100 μm.

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LITERATURE CITED

BAKER, H. B., 1924 ["1923"], Notes on the radula of the Neritidae. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 75: 118–178.

BERTSCH, H. & FERREIRA, A. J., 1974, Four new species of nudibranchs from tropical west America. *Veliger,* 16: 343–353.

BERTSCH, H., FERREIRA, A. J., FARMER, W. M. & HAYES, T. L., 1973, The genera *Chromodoris* and *Felimida* (Nudibranchiata: Chromodorididae) in tropical west America: distributional data, description of a new species, and scanning electron microscopic studies of radulae. *Veliger*, 15: 287–294.

FERREIRA, A. J. & BERTSCH, H., 1975, Anatomical and distributional observations of some opisthobranchs from the Panamic faunal province. *Veliger,* 17: 323–330.

FRETTER, V., 1965, Functional studies of the anatomy of some neritid prosobranchs. *Journal of Zoology, London*, 147: 46–74.

FRETTER, V., 1975, *Umbonium vestiarium*, a filter-feeding trochid. *Journal of Zoology, London,* 177: 541–552.

FRETTER, V. & GRAHAM, A., 1962, *British prosobranch mollusks, their functional anatomy and ecology.* Ray Society, London, xvi + 755 p.

HICKMAN, C. S., 1976, Form, function, and evolution in the archaeogastropod radula. *Geological Society of America, Abstracts with Programs*, 8: 917–918.

HICKMAN, C. S., 1977, Integration of electron scan and light imagery in study of molluscan radulae. *Veliger,* 20: 1-8.

HICKMAN, C. S., 1980, Gastropod radulae and the assessment of form in evolutionary paleontology. *Paleobiology,* 6: 276–294.

HICKMAN, C. S., 1981, Evolution and function of asymmetry in the archaeogastropod radula. *Veliger*, 23: 189–194.

HYMAN, L. H., 1967, *The invertebrates: volume VI, Mollusca I; Aplacophora, Polyplacophora, Monoplacophora, Gastropoda. . . .* McGraw-Hill, New York, etc., vii + 792 p.

KNIGHT, J. B., COX, L. R., KEEN, A. M., BATTEN, R. L., YOCHELSON, E. L. & ROBERTSON, R., 1960, Systematic descriptions (Archaeogastropoda), *In* MOORE, R. C., ed., *Treatise on Invertebrate Paleontology,* Part I, Mollusca 1: 169–310, Geological Society of America and University of Kansas Press.

MCLEAN, J. H., 1981, The Galapagos Rift limpet *Neomphalus*: relevance to understanding the evolution of a major Paleozoic-Mesozoic radiation. *Malacologia,* 21: 291–336.

MORRIS, T. E., 1980, *Morphological and functional dynamics of the rhipidoglossan radula of* Tegula funebralis *(Mollusca: Gastropoda).* Masters thesis, University of California, Berkeley, 145 p.

MORRIS, T. E. & HICKMAN, C. S., 1981, A method for artificially protruding gastropod radulae and a new model of radula function. *Veliger*, 24: 85–90, 1 pl.

PURCHON, R. E., 1968, *The biology of the Mollusca.* Pergamon Press, London, 560 p.

SCHOOLEY, C., HICKMAN, C. S. & LANE, W. C., 1982, Computer graphic analysis of stereo micrographs as a taxonomic tool. *Veliger*, 24: 205–207, 1 pl.

SOLEM, A., 1972, Malacological applications of scanning electron microscopy II. Radula structure and functioning. *Veliger,* 14: 327–336.

SOLEM, A., 1973, Convergence in pulmonate radulae. *Veliger,* 15: 165–171.

SOLEM, A., 1974, Patterns of radular tooth structure in carnivorous land snails. *Veliger*, 17: 81–88.

SOLEM, A. & RICHARDSON, E. S., Jr., 1975, *Paleocadmus*, a nautiloid cephalopod radula from the Pennsylvanian Francis Creek Shale of Illinois. *Veliger*, 17: 233–242.

SOLEM, A. & ROPER, C. F. E., 1975, Structures of Recent cephalopod radulae. *Veliger*, 18: 127–133.

TORR, C. M., 1914, Radulae of some South Australian Gastropoda. *Transactions of the Royal Society of South Australia,* 38: 362–368.

YONGE, C. M. & THOMPSON, T. E., 1976, *Living marine molluscs,* Collins, London, 288 p.

MALACOLOGIA, 1984, 25(1): 161–172

HETEROSEMATISM IN SNAILS

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ABSTRACT

The colour pattern of the shell of many land snails is somewhat or markedly different on the upper side and on the under side of equi-dimensional to depressed shells. In both these and tall-shelled species the mouth and throat of the shell, and in tall-shelled ones the umbilical region, are often distinctively coloured or patterned. These and the underside of globular to discoidal shells are normally hidden when the snail is attached to a substrate. It is suggested that the different coloration shown if the shell is dislodged by a predator and falls with the concealed area upwards is used to mislead the predator. Examples are given of such a biparatite patterning.

Investigation of the attitude assumed when snails of different shapes are dropped suggests that many have a 1:1 chance of showing the upper or the under side. The possible significance of this ratio for the hypothesis is examined, in relation to a predator bearing two hunting images in its mind at once. A classification of such heterosematic behaviour is suggested.

INTRODUCTION

In many species of land snails with globular, trochoid or depressed shells, the colour pattern is markedly different above and below the periphery of the shell. No such difference is usually seen in snails with tall shells, but in these the umbilical region is often distinctively patterned. In shells of all shapes the lip (if any) and sometimes the throat is strikingly coloured, and often the mantle-surface that fills the mouth of the shell when the animal is resting is strongly pigmented. All land snails attach themselves to a substrate by means of mucus secreted at the mouth of the shell, and the equidimensional shells, and often the depressed ones too, sit on a hard substrate in such a way that much of the shell below the periphery is concealed, as is the oral side of the lip, the throat of the shell, and the animal itself. It is somewhat surprising, therefore, that the subperipheral or circumbilical pattern is often bold and striking, and sometimes at least polymorphic when the supraperipheral is not.

In recent years, much work has been done on the visual searching for prey by predators. The predator (usually a bird), after the successful capture of a prey item, forms a hunting image of that item, and will even pass over prey of different aspect to get at more of that type for which it has formed the image (see e.g. Allen, 1972; Manly, Miller & Cook, 1972 for references). Many animals have therefore taken to unpredictable behaviour, such as erratically zigzagging flight, to confuse, or even, by the arousal of conflict in it, to evoke escape reactions from, a would-be predator (references and discussion in Driver & Humphries, 1970; Humphries & Driver, 1970). Humphries and Driver (1967) adopt the term *protean behaviour* from Chance & Russell (1959), defining it as "behaviour that is sufficiently unsystematic to prevent a reactor from predicting in detail the position or actions (or both) of the actor."

It seems not impossible that some at least of these striking patterns normally concealed on snail-shells may function in a similar way to mislead a predator. This paper describes some of the patterns and considers the behaviour of a shell knocked off its substrate by a predator. The frequencies of different attitudes of the fallen shell are examined for different species, and discussed in the light of this suggestion.

DIFFERENTIAL COLOUR AND BANDING PATTERNS

Among tropical land snails with shells of equidimensional to depressed shape, considerable contrast between the colour patterns above and below the periphery is not uncommon. Thus E. von Martens (1867) gives beautiful coloured figures in his account of an expedition to the Orient of such species

as his *Nanina citrina* L. var. *aurantia*, with a broad purple brown band on a buff background above, and a warm reddish brown below with a narrow white edge, and var. *columellaris* with the upper surface warm deep brown, the lower very pale greenish yellow. In *Nanina sulfurata* v. M. the upper side is boldly banded with black on pale yellow, the lower is plain pale yellow. In *Helix bulbulus* Mousson, again the upper side is spirally banded dark brown on pale whitish buff, the lower is plain pale dirty buff except for a dark brown umbilical band and the flared lip is brown and white. In *Helix pyrostoma* Fér. (now a helicostyline) the whole shell is depressed and yellow-brown but the underside is diversified by the brilliant red lip and throat, the aboral side of the lip, which is visible from above being merely brown. In *Helix exceptiuncula* Fér., the upper side is banded black and white or black, white and buff-grey, while the lower side is plain buff-grey. Many other examples could be given from other tropical faunas.

Many shells, of course, are plain brown all over, pale brown, yellow brown or even nearly glassy, as von Martens shows in his plate 12, and not all the brightly coloured shells show in all their varieties a diversity between upper and underside. Exactly the same is true of the European fauna, in which the plain-coloured shells are mainly leaf-litter dwellers or nocturnal; the patterned ones sit out occasionally or habitually in the daytime (Cain, 1977a). Those exposed to intense sunlight are white all over (e.g. *Sphincterochila*) or at least white or mostly white above, but the latter often retain bands on the underside of the shell (see Cain, Cameron & Parkin, 1969 for the variation in Britain of *Helicella itala* (L.)) and these may be polymorphic. Similarly, in the common Mediterranean helicid *Eobania vermiculata* (Müller), which often sits out on rocks or vegetation, the upper side is usually banded, the bands being often partially broken up into mottles, so that a somewhat uniform marbled effect is obtained. The underside, however, is plain white with distinct narrow black bands (Fig. 1), giving a very different effect, which is emphasized by the visible mantle being plain grey, dark grey, or nearly black. This subperipheral pattern varies, with the bands either entire, as just described, or else broken into brown mottles, or represented only by faint lines, and the variation is genetically controlled (Cain, in prep.). Similarly in *Iberus marmoratus* (Fér.), endemic to Gibraltar (Fig. 2), the same patterns and variation are seen, the upper side contrasting even more by its mottling with the emphatic

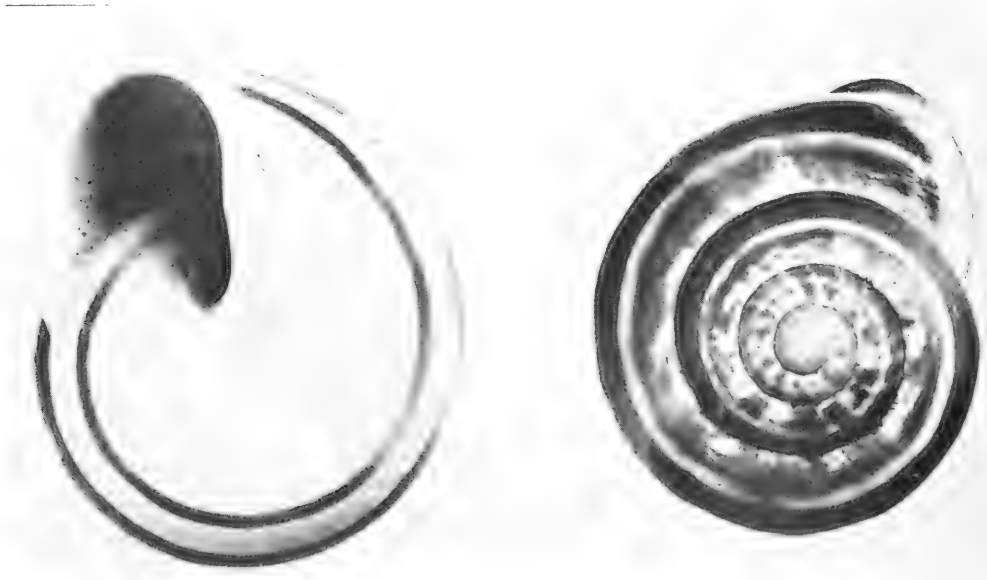

FIG. 1. Shells of *Eobania vermiculata,* 3.3 and 2.9 cm in maximum diameter, showing at left a common morph pattern on the under side of the shell and at right a very common pattern on the upper side.

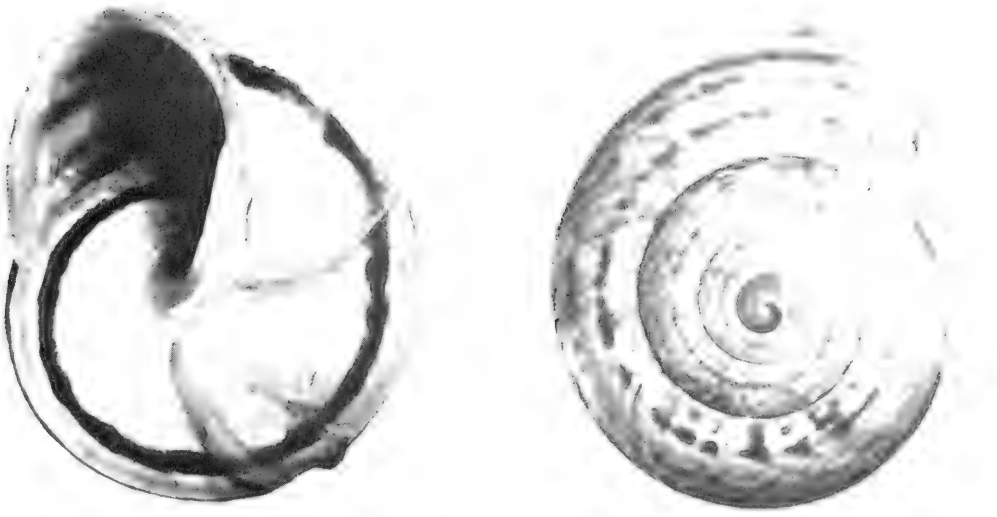

FIG. 2. Shells of *Iberus marmoratus*, 2.2 and 2.1 cm in maximum diameter, showing contrasting patterns on the under side (left) and upper side (right).

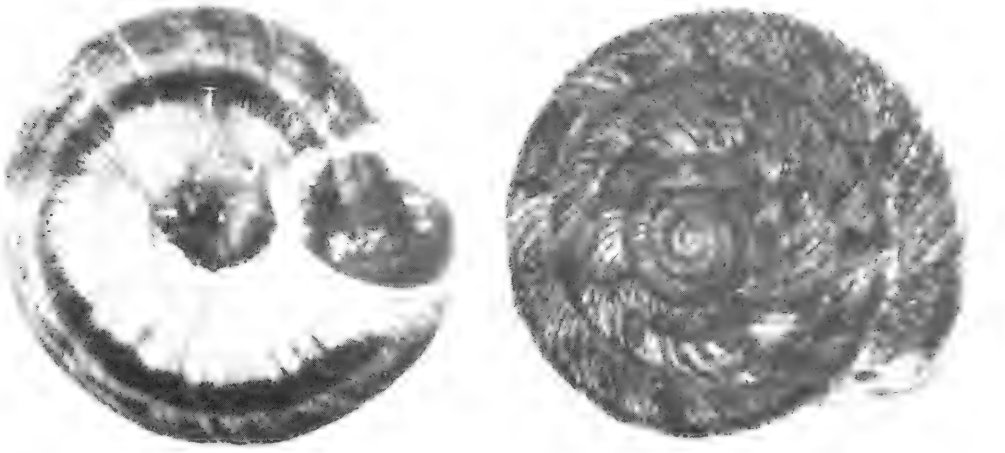

FIG. 3. Under and upper sides of the same shell of *Discula polymorpha*, 1.0 cm maximum diameter.

bands of the underside. In the geomitrine *Discula polymorpha* (Lowe), from Madeira (Fig. 3), the upper side is dark brown and almost uniform, the underside strikingly light with a strong dark band; I have not seen variation in the pattern of the underside in this species.

In the bewilderingly variable snail *Theba pisana* (Müller), the variation in pattern has usually been taken as continuous. Banding may occur all over the shell but is not infrequently more emphatic on either the upper or lower surface; Fig. 4 shows a collection to show range in variation made by Dr. G. Lewis. Some banding forms are genetically controlled (Cain, in prep.; R. H. Cowie, in prep.), and it is usual for them to differ above and below the periphery. Fig. 5 shows the unbanded morph; a form, very well known among helicellines as well as in *Theba pi-*

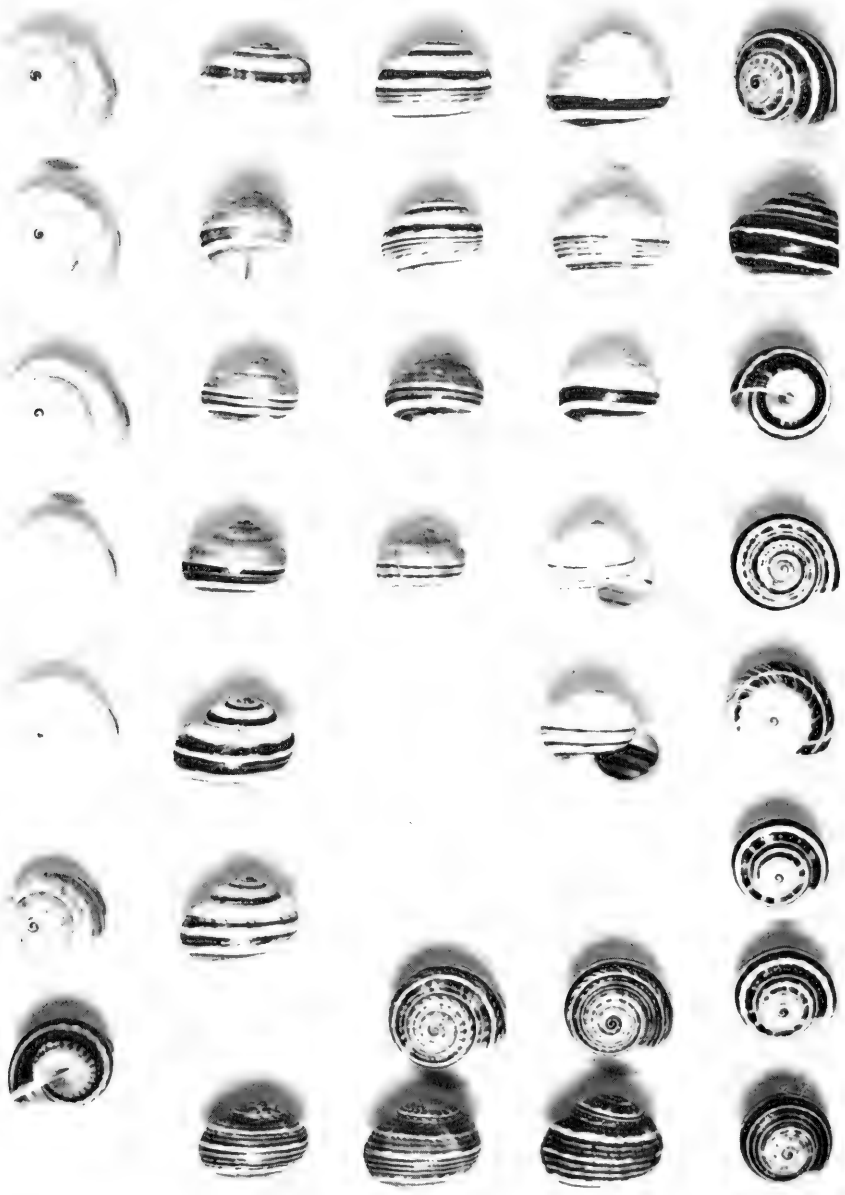

FIG. 4. A range of banding patterns in *Theba pisana*, giving only a small selection of variation in the species.

sana, with a white shell with a strong black peripheral band above and broken bands below; a form with yellowish blotches and black dashes above, darkening on the body whorl, but with a thin black band below, or none; and a more conventionally five-banded form in which the dottings and dashes of the upper bands on the spire contrast with the broad nearly plain umbilical area which is emphasized by strong bordering bands.

In *Cepaea nemoralis* (L.) and *C. hortensis* (Müller), an unbanded morph and one with a single peripheral band (00300) are well-known, though the latter is very rare in *C. hortensis*. In both these morphs, the shell appears plain (yellow, pink or brown) both

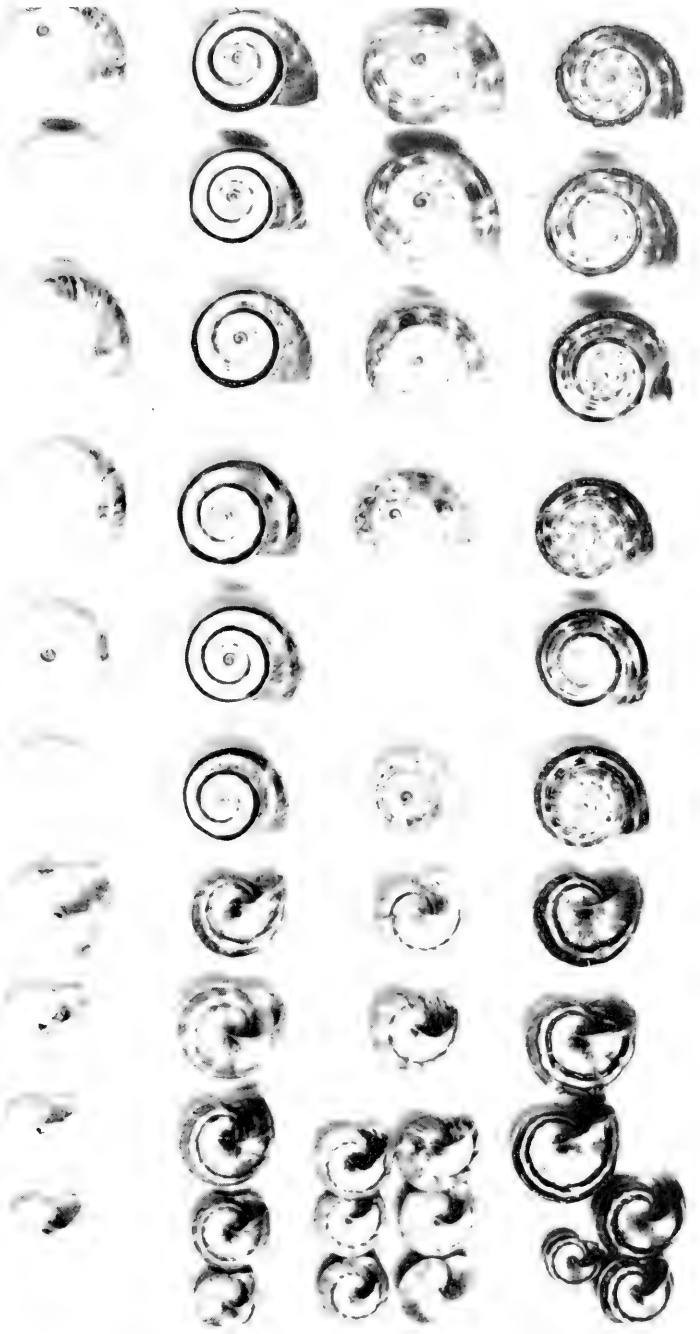

FIG. 5. Morphs of *Theba pisana.*

above and below, but from below, the white or black-brown lip and callus and the animal's mantle are conspicuous features. All the possible combinations of presence or absence of the five bands, and fusion of adjacent bands appear to have been found, at least in *C. nemoralis,* but there is an extreme rarity of forms with bands above but not below the periphery, while those with bands below but not above are quite common (Taylor, 1910).

Thus such forms as 00345, 00(34)5, 00(345), 00:45, 00:(45), and 00045 (the parentheses indicate fusion of the contained bands) are far from uncommon, whereas 12300 is extremely rare. It is known that visual predation can exert selection upon the morphs of *Cepaea* such that heavy banding is at an advantage in very stripy habitats, lack of banding (00000) and effective lack of banding in the normal position of a resting snail (00300, 00345 etc.) in more uniform ones. It is also highly likely that in regions of strong insolation, the upper part of the shell at least must be light-coloured (yellow and effectively unbanded) to reflect back as much incident radiation as possible (see Jones, Leith & Rawlings (1977) for a review).

Insolation will affect the underside of the shell less; it therefore can be banded or not. Under visual selection, it may be necessary to be banded all over, and indeed the fully-banded morph, 12345, is common in both species. But it is noticeable even in this morph that the banding is very different on the upper and under sides (Fig. 6), and that each band has its characteristic position and breadth. It was the highly individual characters of the bands of *C. nemoralis* that led G. von Martens (1832) to the realization that the variation was not merely random, and to the present system of their nomenclature. Bands 1 and 2 are thin and narrow, 3 wider, 4 the widest, and 5 somewhat variable but broad like 3. As seen from above, therefore, the five-banded form shows 3 narrow dark bands (band 3 being foreshortened) on a paler background. From below (apart from the lip, callus and mantle) one sees a large area of pale shell colour emphatically bordered by two large black bands very close to each other (and often fused together). The pattern above is rather light and stripy; below, it is of larger

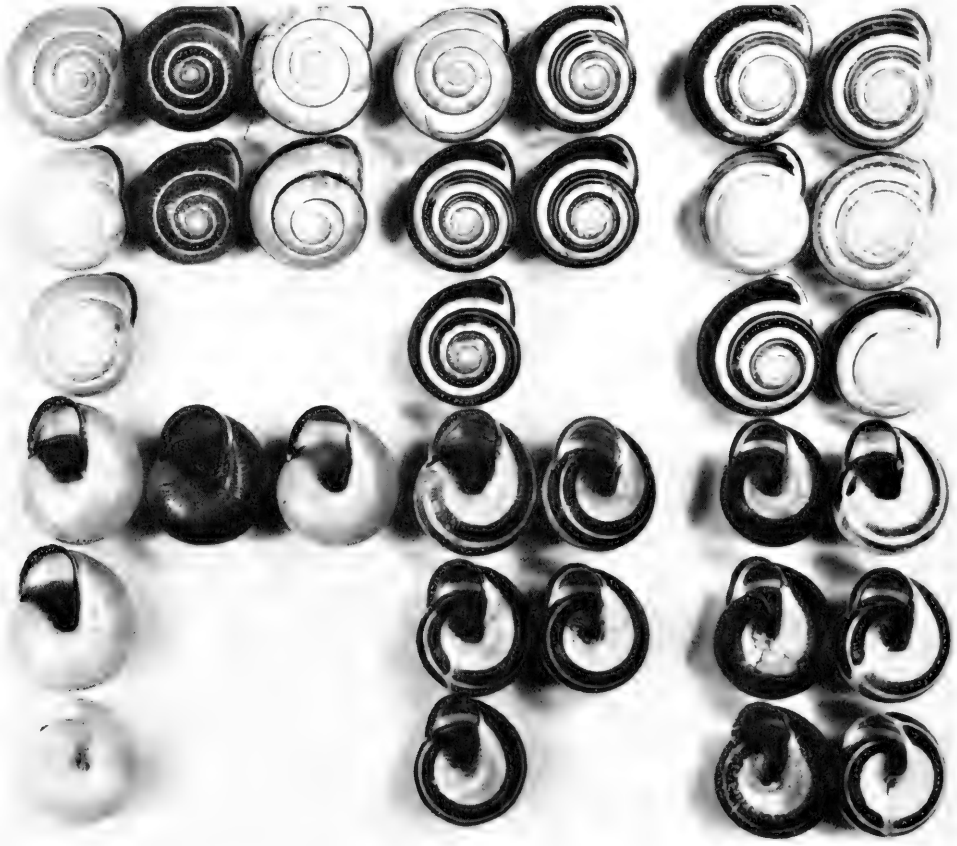

FIG. 6. Variation in banding within a single random sample of a population of *Cepaea nemoralis*; shells within each principal morph in the sample selected at random to show the upper or the under side.

plainer areas, much as in the five-banded morph of *Theba pisana* (Fig. 5). The visual effect, therefore, although banded in both aspects, is markedly different.

It seems worthwhile asking, therefore, whether even when under the constraint of visual selection for general crypsis there is a difference of pattern above and below such that if a snail were to be dislodged by predators and fall base upwards, there might be a chance of it appearing different enough to be overlooked. Certainly, the remarkable differentiation of the bands needs explaining. When in many species of terrestrial snails (e.g. in the helicostylines and oreohelicids) very numerous bands, not groupable into the standard five of the helicids, can be seen, it can hardly be suggested that there is any inherent necessity for the bands of *Cepaea* to be so individually characterized. Moreover, the genetic control of the banding is not by individual bands but by whole patterns at a time; the principal banding morphs are not 10000, 02000, etc., but 12345, 00345, 00300 and 00000. Pettitt (1973) in proposing a mode of scoring the morphs of species of *Littorina* modelled on that for *Cepaea* has overlooked this important consideration. Similarly, in *Theba pisana* with such a remarkable variety of bands and bandlets, one needs to find an explanation of why certain banding combinations giving different appearances above and below the periphery are clipped together genetically.

The measurement of actual selection coefficients in the field is often extremely difficult for a number of reasons (Cain, 1977b), and failure to show differential predation may have no significance. One can ask, however, what consequences are likely to follow from the hypothesis outlined above. If snails are seen by a predator crawling or sitting on a substrate, and are recognized, seized and eaten, then the only selection exerted by the predator is likely to be for some sort of crypsis, unless the prey is so distasteful that conspicuousness is advantageous. This has not been suggested, to my knowledge for snails (though it may be true of some slugs). Nor are snails known as mimics of distasteful animals. (Certainly, bird predators generalize their experiences sufficiently far for that.) If an attached snail is dislodged and drops to the ground, it has a possibility of misleading the predator if it shows a different pattern on its under side, which was till then concealed. Only discoidal or depressed shells have an obvious upper and under-side; globular ones have no plane of even approximate geometrical symmetry, and the distribution of the organs of the body in the shell, with possible differences locally in specific gravity, do not seem to have been investigated. How do snails land when they fall and roll or bounce?

Furthermore, it is evident that tall shells, with height much greater than maximum diameter, can only lie on their side when they fall, and no distinction between patterns above and below the periphery can be made. Very few shells in this position show any dorsoventral flattening; the few that do are plain shells, without any pattern or with undifferentiated mottling, probably lurkers in leaf-litter. The vast majority have no flattening and, unlike many of the equidimensional to discoidal shells, they have no differentiation of the pattern which is the same on whatever side they lie, with one important exception. This is the mouth and throat, and not infrequently the umbilical region. It is a question, therefore, whether the asymmetry of the mouth does compel the shell to lie in a mouth-up or mouth-down position.

POSITIONS OF REST

Tests

Table 1 shows the species of live snail available for investigating this question. A single individual was used of each, since their shells differed markedly in shape, size and weight, except that three individuals of *Helix aspersa* Müller differing in spire index were used, and an adult and juvenile of *Theba pisana*.

Each snail was left dry for a day or more so that it would be retracted into the shell but filling it more or less to the mouth. One *H. aspersa* was tested after several weeks of drought and was so far back in its shell that about a quarter of the body-whorl was empty, but the results obtained with it hardly differed from those after it was fed and watered and brought into the same position as the others. For equidimensional and depressed shells, each individual was held at 20 cm above a flat cloth spread on a hard bench-top, adjusted so that its spire was uppermost, in both cases with the columella vertical, or it was placed in the broad side down position with the columella horizontal and the outer edge of the mouth uppermost. It was then dropped by

TABLE 1. Systematic positions, names, and physical characters of the individuals used in the experiments.

	Weight (gm)	Height mm	Diameter mm	h/d
Helicidae				
Helicinae				
Helix aspersa (Müller) 1	3.80	23.3	27.1	0.86
Helix aspersa 2	4.48	26.3	27.3	0.96
Helix aspersa 3	8.99	34.4	30.6	1.12
Eobania vermiculata (Müller)	5.73	20.1	27.6	0.73
Pseudotachea splendida (Draparnaud)	1.40	10.8	19.5	0.55
Alabastrina alabastrites (Michaud)	2.61	13.4	21.4	0.63
Levantina caesareana (Parreyss)	6.60	23.2	30.5	0.76
Theba pisana (Müller) adult	2.52	15.3	19.4	0.79
Theba pisana juvenile	0.13	5.1	8.1	0.63
Helicigoninae				
Dinarica pouzolzi (Férussac)	16.73	24.6	40.0	0.62
Leptaxinae				
Leptaxis undata (Lowe)	4.07	15.5	25.2	0.62
Geomitrinae				
Discula polymorpha (Lowe)	0.25	5.5	10.1	0.54
Helminthoglyptidae				
Monadenia troglodytes Hanna & Smith	2.01	11.5	22.6	0.51
Zonitidae				
Oxychilus cellarius (Müller)	0.14	3.0	7.8	0.38
Subulinidae				
Rumina decollata (L.)	2.27	26.4	12.2	2.16
Clausiliidae				
Papillifera bidens (L.)	0.09	13.8	3.8	3.63
Bulimulidae				
Placostylus porphyrostomus (Pfeiffer)	26.47	69.4	32.9	2.11
Placostylus fibratus (Martyn)	11.28	54.6	25.5	2.14

parting the fingers with a jerk; usually, on hitting the cloth it had enough energy to bounce and/or roll. Its final position was recorded as with apex up or down. For tall shells, only the apex up and apex down positions were used at release; positions of rest were scored as with mouth up (u), mouth down (d), on its side with the umbilical area above the mouth (s), on its side with the outer lip uppermost (s′), and intermediate positions (e.g. s′u, su) as required. The whole procedure for both types of shell was repeated from 10 cm above the cloth. Small and light shells (e.g. *Oxychilus*, juvenile *Theba, Discula*) withstood the tests well, as did large and heavy but thick ones (*Placostylus*). As 20 observations were taken at every position, each shell had a possible total of 360 impacts. It is perhaps not surprising that the large but rather thin-shelled *Helix aspersa* could not withstand this battering and chipped or cracked before the full set was completed. (An *Otala punctata* cracked at the first drop from 20 cm.) The *Monadenia* was given only a partial test.

Results

Table 2 gives the actual results for near-equidimensional to depressed shells, Table 3 for tall ones. The results of χ^2 tests for heterogeneity of data within or between runs for each species, and for departure from 1:1 ratio on the data in Table 2 are given in Table 4. For all but three of the individual snails there is no heterogeneity in the results, and for eight no significant departure from a 1:1 ratio. For the *Helix aspersa* 2 and 3, the shell rested more often with mouth up and apex down than would be expected if the ratio were 1:1, and the same was true of the *Oxychilus,* but for the *Leptaxis undata* and for *Theba pisana,* especially the juvenile, the departure was in the opposite direction, the shell resting more often with the apex up. The peculiar shape of the juvenile *Theba pisana,* with almost flat upper surface and strongly rounded underside suggests that the other way up would be most stable, but with the apex up, it rolls around like a top without reversing its position. Of those showing heterogeneity in

TABLE 2. Resting positions of individuals dropped from different heights and initial attitudes. In each pair of figures, the first is the number of times the snail came to rest with apex up (and mouth down), the second with apex down (and mouth up).

	Height and initial attitude						
	20 cm			10 cm			
Species	Apex up	Apex down	Broad side down	Apex up	Apex down	Broad side down	Total
Helix aspersa 1.	32/28	31/29	11/9	32/27	30/30	–/–	137/123
Helix aspersa 2.	15/25	18/22	–/–	3/17	6/14	–/–	42/78
Helix aspersa 3.	16/44	–/–	–/–	35/25	24/36	22/38	97/143
Eobania vermiculata	28/32	21/39	33/27	25/35	28/32	27/33	162/198
Levantina caesareana	27/33	33/27	22/38	31/29	36/24	31/29	180/180
Alabastrina alabastrites	38/22	33/27	27/33	31/29	33/27	25/35	187/173
Pseudotachea splendida	32/28	33/27	26/34	23/37	33/27	30/30	177/183
Theba pisana adult	34/26	30/30	34/26	33/27	34/26	34/26	199/161
Theba pisana juvenile	40/20	39/21	34/26	34/26	39/21	37/23	233/137
Dinarica pouzolzi	27/33	32/28	32/28	36/24	47/13	21/39	195/165
Leptaxis undata	36/24	32/28	29/31	42/18	42/18	30/30	211/149
Discula polymorpha	24/36	24/36	33/27	32/28	27/33	35/25	175/185
Monadenia troglodytes	22/38	24/36	–/–	31/29	31/29	–/–	108/132
Oxychilus cellarius	24/36	27/33	22/38	22/38	27/33	31/29	153/207

the results, *Helix aspersa* 3 showed marked departures when dropped from 20 cm and 10 cm with apex up, but the departures are in opposite directions. The significance (if any) is unclear. In the case of *Dinarica pouzolzi* (Fér.) the reasons are obvious. This is a large heavy broad snail, and when dropped from only 10 cm tended to keep its orientation. (Its' one-sided results when dropped broad side down may point to some overall asymmetry, but this is the position least easy to standardize with accuracy). The *Leptaxis* showed a strong tendency to rest with apex up when dropped from 10 cm in both the apex-up and apex-down positions, as well as an overall trend in this direction. There may be a real bias here.

Of the species investigated, *Leptaxis undata* (Lowe) and *Oxychilus cellarius* (Müller) are fairly uniformly brown; the rest have different bands above and below, the difference being most striking in *Discula polymorpha* (Lowe), *Eobania vermiculata* (Müller) and (often) *Theba pisana*. An overall χ^2 test for departure from 1:1 on the totals for each specimen is still highly significant at the 95% level (d.f. 13, $\chi^2 = 72.9$) as is an overall heterogeneity test on all the runs of observations, usually six for each specimen but sometimes less (d.f. 76, $\chi^2 = 173.8$).

In Table 3, it can be seen without statistical tests that the tall shells also have a strong bias to resting with the mouth definitely up (u) or nearly so (su) or definitely down (d) but that intermediate positions are not uncommon. It might well be thought that a small light form such as the little clausiliid *Papillifera bidens* (L.) might be held in any position by the roughness of the soft cloth onto which the snails were allowed to fall, as a larger shell might be on grass, and that the frequency of four positions merely reflects the natural tendency of the observer to overclassify a continuum of position into four quadrants. This, however, is not the case; if the shell is placed on a sheet of plastic which can be vibrated gently with the fingers, to test the stability of any given position, it is found that there are four principal positions, although those with mouth up and mouth down are more readily retained than the other two. In the case of the *Placostylus,* these heavy forms with somewhat irregular shells can take a greater variety of positions which depend on the exact shape of those parts of the shell resting on the substrate. Even these tend to rest most frequently with mouth down or up, or nearly up (su) in *P. porphyrostomus* (Pfeiffer). Of the tall shells, *Rumina decollata* (L.) and *Papillifera bidens* are plainly coloured. (*P. bidens* has a reddish-brown band along the suture ornamented with whitish dots, but this

TABLE 3. Resting positions, after dropping, of tall-shelled snails. For explanation of the positions, see text.

	Height and initial attitude	u	su	s	s'u	s'	d
Rumina decollata							
	20 cm apex up	23	—	4	—	9	24
	apex down	27	—	4	—	12	17
	10 cm apex up	20	—	4	—	18	18
	apex down	20	—	6	—	15	19
		90	—	18	—	54	78
Papillifera bidens							
	20 cm apex up	12	—	16	—	11	21
	apex down	16	—	14	—	13	17
	10 cm apex up	22	—	13	—	14	11
	apex down	13	—	13	—	18	16
		63	—	56	—	56	65
Placostylus porphyrostomus							
	20 cm apex up	14	28	—	—	—	18
	apex down	6	20	—	1	—	33
	10 cm apex up	9	30	—	2	1	18
	apex down	6	27	—	—	—	27
		35	105	—	3	1	96
Placostylus fibratus							
	20 cm apex up	12	2	3	—	1	42
	apex down	15	4	2	1	2	36
	10 cm apex up	10	—	2	—	2	46
	apex down	19	1	4	3	2	60
		56	7	7	4	11	155

TABLE 4. Tests of heterogeneity between runs for each individual used, and for departure of the ratio of the resting positions from 1:1.

	Heterogeneity				
	d.f.	χ^2		Departure from 1:1	(d.f.1)
Helix aspersa 1	4	0.76		0.38	
Helix aspersa 2	3	5.60		10.80	
Helix aspersa 3	3	13.06	*	8.82	*
Eobania vermiculata	5	5.25		3.60	(near)
Levantina caesareana	5	8.00		none	
Alabastrina alabastrites	5	7.27		0.54	
Pseudotachea splendida	5	5.70		0.33	
Theba pisana adult	5	0.87		4.01	*
Theba pisana juv.	5	2.46		20.54	*
Dinarica pouzolzi	5	25.88	*	2.50	
Leptaxis undata	5	11.60	*	10.68	*
Discula polymorpha	5	7.66		0.28	
Monadenia troglodytes	3	4.44		2.40	
Oxychilus cellarius	5	4.19		8.10	*

*Indicates significant difference at the 95% level.

appears to be a breaking-up of the regular line of the suture—compare Cott, 1940 p. 110—and, being continuous, is not related to attitude.) The two species of *Placostylus* have dark throats, richly coloured in *P. porphyrostomus,* bordered by prominent pale lips, but the body of the shell is fairly uniform in colour.

DISCUSSION

If the underside of depressed shells or the oral-umbilical area in tall ones were invariably shown when the animal fell and the coloration was vivid in comparison with that of the upper side or the main cone of the shell respectively, the result would be a sort of flash coloration but with the disadvantage that it could not be concealed again rapidly, the animal returning to a cryptic pattern. The upper side of such forms as *Iberus marmoratus* or *Discula polymorpha* is indeed more cryptic than the underside, but in some morphs of *Cepaea* (e.g. red 00045) and some of the species figured by E. von Martens neither aspect appears particularly cryptic, only noticeably different from the other aspect. In other morphs of *Cepaea* both aspects may be cryptic (e.g. yellow 12345) but still differ noticeably. It seems reasonable to suggest, therefore, that the difference is used for misleading the predators, not as warning coloration or a different kind of crypsis. The narrow black bands on a pale ground on the underside of *Eobania vermiculata, Iberus marmoratus* or *Discula polymorpha* are hardly cryptic yet unlike the heavily banded and mottled or dark brown upper surface. It seems unlikely that selection for crypsis could cause such differences; and selection against insolation should merely pale the upper surface, leaving the lower unaffected.

None of the shells investigated rested invariably, or even nearly so, with the normally concealed parts exposed; on the contrary, ratios from about 1:2 to about 2:1 seem nearly always to hold, not 1:0 nor any approximations to it. Equal or subequal tendencies to show the upper and under sides are seen not only in those shells with contrasting colour patterns but also in those which are plain. This property must be a function of the shape of the shell, not at all associated with a particular style of patterning, and there is little indication that the disposition of the body in the shell causes much bias, although this needs further examination. Preliminary runs with an empty shell of *Dinarica pouzolzi* gave very similar results to those with a full shell, and tests with one of *Placostylus porphyrostomus* on a gently agitated tympanum (as described above for *Papillifera bidens*) gave the same positions of stability as with the live animal. Furthermore, land snail shells do not have projecting wings or vanes, so that orientation by the medium through which they fall (air) will not occur, in contrast to the marine shells investigated by Palmer (1977).

Exactly what determines the shape of any terrestrial gastropod's shell is as yet quite uncertain, and it would not be reasonable to suggest that any of these shells have evolved primarily to fall in one of two opposite positions. But just as haemoglobin was surely evolved for its oxygen-carrying properties, yet its concomitant redness can be used in blushing (Goodhart, 1960), so the shape of the shell, dictated by other considerations, may be used in whatever further ways it permits. To take the most frequent case so far, what will be the effect on a predator if the shell is equally likely to fall with the mouth up or the mouth down (in depressed shells with apex down or apex up respectively) and the colour patterns of the two aspects are different? This can be answered only by experimental observation, but it can be suggested that an arrangement which maximises the number of times the same aspect is shown before and after falling will strengthen the primary hunting image of it. One which maximises the number showing the different aspect *and* thereby misleading the predator will be most strongly selected for, but the success of the device will depend on the strength of reinforcement of the primary hunting image and the unpredictability of what will be shown on each occasion. In fact, a random fall producing one of two markedly different images in an overall ratio of 1:1 may be the most effective strategy as in the game "which hand is it in?" (Driver & Humphries, 1970).

Furthermore, since the underside pattern is normally hidden, it is free to vary, at least as far as visual selection for crypsis and selection against heating by insolation are concerned. As already mentioned, there is a series of genetically determined variations of the under-side pattern in *Eobania vermiculata* and *Iberus marmoratus* which leave the upper-side pattern much the same. Relative variability in under-side patterns needs more investigation in other species, such as *Cepaea nemoralis* and *Theba pisana*.

Humphries and Driver (1970) include visible polymorphism in the shell of *Cepaea* under their heading of protean behaviour. Mimicry, however, they include (p. 286) under the heading of regular devices in predator/prey strategy, as against the irregular devices which are characteristic of protean behaviour, but as an example of mimicry they refer to Sheppard's work which is on polymorphic mimicry. The status of polymorphic populations in their classification is therefore not clear. Their application of Chance and Russell's term *protean* to many sorts of highly complex sequences of behaviour seems excellent, but there are two groups of behaviours which connect protean to regular behaviour, namely (i) polymorphic mimicry in which each individual has only one constant signal-pattern but this varies from individual to individual, and (ii) such examples as the snails dealt with here are suggested to be. In these, a population may be monomorphic or polymorphic but some, if not all, of the individuals have two distinctly patterned aspects. While recognizing that there is almost certainly a continuum, I would prefer to keep protean for behavior which is indeed protean and use the less specific term *heterosematic* (signalling different things) for less complex misleading behaviour.

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LITERATURE CITED

ALLEN, J. A., 1972, Evidence for stabilizing and apostatic selection by wild blackbirds. *Nature, London,* 237: 348–349.

CAIN, A. J., 1977a, The uniqueness of the polymorphism of *Cepaea* (Pulmonata: Helicidae) in western Europe. *Journal of Conchology,* 29: 129–136.

CAIN, A. J., 1977b, The efficacy of natural selection in wild populations. *In* GOULDEN, C. (ed.), *The changing scenes in natural sciences 1776–1976. Academy of Natural Sciences Special Publication,* 12: 111–133.

CAIN, A. J., CAMERON, R. A. D. & PARKIN, D.T., 1969, Ecology and variation of some helicid snails in northern Scotland. *Proceedings of the Malacological Society of London,* 38: 269–299.

CHANCE, M. R. A. & RUSSELL, W. M. S., 1959, Protean displays: a form of allaesthetic behaviour. *Proceedings of the Zoological Society of London,* 132: 65–70.

COTT, H. B., 1940, *Adaptive coloration in animals.* Methuen, London, xxxii + 508 p., 48 pl.

DRIVER, P. M. & HUMPHRIES, D. A., 1970, Protean displays as inducers of conflict. *Nature, London,* 226: 968–969.

GOODHART, C. B., 1960, The evolutionary significance of human hair patterns and skin colouring. *Advancement of Science,* 17: 53–59.

HUMPHRIES, D. A. & DRIVER, P. M., 1967, Erratic display as a device against predators. *Science, N.Y.,* 156: 1767–1768.

HUMPHRIES, D. A. & DRIVER, P. M., 1970, Protean defence by prey animals. *Oecologia, Berlin,* 5: 285–302.

JONES, J. S., LEITH, B. H. & RAWLINGS, P., 1977, Polymorphism in *Cepaea*: a problem with too many solutions? *Annual Review of Ecology and Systematics,* 8: 109–143.

MANLY, B. F. J., MILLER, P. & COOK, L. M., 1972, Analysis of a selective predation experiment. *American Naturalist,* 106: 719–736.

MARTENS, E. VON, 1867, *Die preussische Expedition nach ost-Asien.* Zoologischer Theil. v. Decker, Berlin, 2: i–xii, 1–447, 22 pl.

MARTENS, G. VON, 1832, Über die Ordnung der Bänder an den Schalen mehrerer Landschnecken. *Deutsche Akademie der Naturforscher, Nova Acta physicomedica (Nova Acta Leopoldina)* 16: 177–216.

PALMER, A. R., 1977, Function of shell sculpture in marine gastropods: hydrodynamic destabilization in *Ceratostoma foliatum. Science, N.Y.,* 197: 1293–1295.

PETTITT, C., 1973, A proposed new method of scoring the colour morphs of *Littorina saxatilis* (Olivi, 1792) (Gastropoda: Prosobranchia). *Proceedings of the Malacological Society of London,* 40: 531–538.

TAYLOR, J. W., 1910, *Helix nemoralis* Linné. *Monograph of the Land and Freshwater Mollusca of the British Isles,* part 17: 274–325.

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PATTERNS OF DIGESTIVE TRACT MORPHOLOGY IN THE LIMACISATION OF HELICARIONID, SUCCINEID AND ATHORACOPHORID SNAILS AND SLUGS (MOLLUSCA: PULMONATA)

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ABSTRACT

The posterior part of the digestive tract of most Stylommatophora becomes hypertorted some 180° more during ontogeny than is seen in the post-torsional embryo. In a family of helicoid snails such as the Helicarionidae *s.l.*, this hypertorsion is retained during the initial steps of limacisation, and may be retained or lost in semislugs and slugs. The process by which the stomach is included in the foot cavity in the last steps of limacisation depends on the position of the upper posterior edge of the foot cavity, which in a semislug may be either behind or in front of the stomach. As a result there are four main processes by which the digestive tract of a helicoid snail may be integrated into the foot cavity. Some slugs originating from helicoid snails have exaggerated hypertorsion of their digestive tract.

In succineids, the digestive tract is detorted in the course of limacisation. This detorsion may be related to their bulimoid shell shape.

Athoracophorids have more exaggerated hypertorsion of the digestive tract than any other slug. Their peculiar anatomical characters may result from advanced limacisation. In particular, their lung may result from the development of structures that occur as accessory structures in the lung of some unrelated slugs. It is concluded that athoracophorids are more closely related to other Aulacopoda than to succineids.

INTRODUCTION

Although the digestive system occupies nearly the whole body and visceral cavity of the Stylommatophora, there are few data on this system (cf. Runham, 1975). Its description takes only about twenty pages of Simroth & Hoffmann's monumental 1354-page work (1908–1928). In most of the few general drawings scattered in the literature, the position of the tract has been modified to show as much as possible of its surface. Furthermore, as the internal morphology generally is not described, it is impossible to propose meaningful comparisons among taxa. An example is Rigby's statement (1965) that succineids are terrestrial opisthobranchs, proved erroneous by Solem (1978). As the digestive system of the Stylommatophora is relatively big, its comparative anatomy is very important in the study of *limacisation*, the process by which a snail with a helicoid visceral hump evolves into a slug without a prominent visceral hump.

The systematic position of two stylommatophoran families including slugs or semislugs, the Succineidae and Athoracophoridae, has been controversial for more than a century, as summarized by Van Mol (1967) and Solem (1978). Solem places these two families among the Aulacopoda, an infraorder of sigmurethrous Stylommatophora that surprisingly includes helicoid snails and slugs, but no bulimoid snails other than succineids. The purpose of this paper, mainly based upon comparative gross anatomy of the digestive tract, is to show: (1), how the digestive tract of the Aulacopoda becomes entirely contained in the foot cavity (example using the helicarionids *s.l.* [= most of Ariophantacea auct.]); and (2), new insights into systematic relationships resulting from a comparison of these stages with the situation found in the families Succineidae and Athoracophoridae. To choose the helicarionids as a basis for comparison is justified not only by their unrivalled wealth in semislugs, but also because, like succineids and athoracophorids, they show nearly complete absence of internal ridges in the stomach. The central nervous system and the pallial complex, which have been used to provide major taxonomic characters, have also been employed.

MATERIALS AND METHODS

All animals mentioned in this paper are preserved in alcohol in museum collections (Table 1). To show the gross morphology of the digestive tract, the outline of the animals was first drawn with a camera lucida. The slugs and bulimoid snails were drawn in dorsal view, the columellar axis parallel to the drawing plane; the helicoid snails were drawn seen from the apex of the shell, the columellar axis perpendicular to the drawing plane. The shell, when present, was removed or dissolved in acid and the snail or slug progressively dissected. Each time a part of the digestive tract was uncovered by dissection it was drawn under a camera lucida with the animal positioned as described above. Finally, when the digestive tract had been entirely drawn *in situ*, it was opened longitudinally to observe its internal morphology, which was depicted in the same drawing as the external morphology. For all drawings, the rectum was cut off when removing the lung during dissection; its position along the suture of the body whorl is the same for all snails. No live specimen of any species studied could be obtained, and thus it was impossible to observe ciliary currents and food transport. Histological study of the digestive tract has not been attempted, since it seems more urgent to define the gross morphology first.

A few sections of lung were made but not illustrated; they were also prepared from animals preserved in alcohol. The lung was refixed in Bouins and the sections stained with Masson's trichrome.

TABLE 1. List of specimens cited in this paper (MNHN = Muséum national d'Histoire naturelle, Paris; MRAC = Musée Royal de l'Afrique Centrale, Tervuren).

Athoracophoridae
- Athoracophoridae n. sp., Valley of Amoa, New Caledonia, Tillier coll. 18.1.1981, MNHN.
- *Aneitea simrothi* Grimpe & Hoffmann, Thiem, New Caledonia, Bouchet coll. 25.12.1978, G. M. Barker det., MNHN.

Succineidae
- *Succinea putris* (L.), Le Hade (Seine maritime), France, Chevallier coll. 29.9.1967, MNHN.
- *Succinea propinqua* Drouët, Ilet la Mère, French Guyana, F. Geay coll., MNHN.
- *Hyalimax perlucidus* (Quoy & Gaimard), St-Philippe forest, La Réunion, Lantz coll., MNHN.
- *Omalonyx matheroni* (Potiez & Michaud), between Cayenne and Kourou, French Guyana, Tillier coll. 1978, MNHN.

Helicarionidae
- Helicarioninae (?)
  - *Kalidos oleatus* (Ancey), Marojezy, W from Sambava, alt. 1300 m, Madagascar, 8.12.1972, Fischer-Piette det., MNHN.
  - Helicarioninae sp., Poindimié, New Caledonia, Bouchet coll. 29.12.1978, MNHN.
  - *Malagarion paenelimax* Tillier, holotype, Marojezy, alt. 600 m., Madagascar, Blanc coll. 12.1972, MNHN.
- Ariophantinae (Parmarioni)
  - *Parmarion* sp., Cambodia, Harmand coll. 1898, MNHN.
- Ariophantinae (Girasii)
  - *Mariaella dussumieri* Gray, Mahé, probably southern India, Dussumier coll. 1835, MNHN.
- Gymnarioninae (?)
  - *Gymnarion sowerbyanus* (Pfeiffer), Assinie, Ivory coast, Chaper coll. 1882, MNHN.
- Urocyclinae (?)
  - *Granularion lamottei* Van Mol, Mt Nimba, Guinea, Lamotte coll. 1956, MNHN.
  - *Estria?* sp. A (Van Goethem, 1977), Gopoupleu, Ivory Coast, Condamin and Roy coll. 1959, MNHN.
  - *Tresia parva* Van Goethem, paratype, Mt Nimba, Guinea, Lamotte et al. coll. 1957, MNHN.
  - *Elisolimax madagascariensis* (Poirier), Montagne d'Ambre, Madagascar, Blanc & Salvat coll. 1970, MNHN.
  - *Atoxon pallens* Simroth, Virunga park, Zaïre, Vanschuytbroek coll. 1954, Van Goethem det. et leg., MNHN.
  - *Mesafricarion maculifer* Pilsbry, Lodjo, Mongbwalu, Zaïre, Lepersonne coll. 7.1939, Van Mol det., MRAC.

Parmacellidae:
- *Parmacella* sp., Algeria, M. Marès coll. 1876, MNHN.

GENERAL CHARACTERS OF THE DIGESTIVE TRACT OF THE STYLOMMATOPHORA

Gross anatomy of the digestive tract

In nearly all Stylommatophora, the most posterior region of the digestive tract, equivalent to the uppermost region in a coiled visceral mass, is the stomach, which is defined as the part of the digestive tract that receives the two ducts of the digestive gland. Between the mouth and the stomach are the buccal mass, the oesophagus and the crop; beween the stomach and the anus is the intestine. The anus opens on the right side of the body, except in sinistral species, and generally close to the pneumostome.

The oesophagus always begins at the posterior upper aspect of the buccal mass. In carnivorous snails and slugs, the part of the buccal bulb behind the opening of the oesophagus is secondarily elongated backward to form an evaginable snout (e.g. Watson, 1915), but this is not the case in any species studied here. The oesophagus generally has internal longitudinal ridges. In the families studied here, these ridges, if present, generally are thin. In some species only two ridges are present (*Elisolimax*, Fig. 12), or ridges are absent (*Aneitea simrothi* Grimpe & Hoffmann, Fig. 20). The oesophagus passes posteriorly to an inflated crop. The term oesophagus is ambiguous for two reasons: (1) In most holopod snails and some Holopodopes (classification of Solem, 1978), the oesophagus itself is inflated into a crop separated from the inflated region immediately anterior to the stomach by a section of typically folded oesophagus (Tillier, unpublished). This may be an oesophageal crop, and thus not homologous with the crop of such snails as *Achatina*, which Ghose (1963) claimed is of gastric origin. (2) The part of the digestive tract between oesophagus and stomach is sometimes divided into two inflated pouches separated by a constriction (cf. *Gymnarion*, Fig. 4, and *Malagarion*, Fig. 3). They are here called the anterior and posterior crop. It is unknown if the anterior crop is formed by dilatation of a section of oesophagus or if it is homologous with the anterior part of the crop of the snail ancestor, brought to this position by shortening the oesophagus during limacisation. In many species the transition from oesophagus to crop is gradual. It is incorrect to assume the oesophagus is shorter than the crop in all Stylommatophora (Runham, 1975): this paper shows that shortening of the oesophagus is the result of limacisation. In families studied here the crop has at most a few internal ridges which are possibly secondary. No species of these families examined has two longitudinal ventral ridges leading to the stomach, as found in *Oxychilus* (Rigby,1963).

In nearly all snails and slugs, the stomach is the part of the digestive tract extending farthest from the mouth, forming a bend from which the intestine goes forward. In no family studied here is the stomach clearly differentiated from the crop, inside or outside. It appears to be a simple end of the crop receiving the two ducts of the digestive gland. As will be discussed later, the intestine opens from the lower columellar side of the stomach of most snails, and from the right lower side of the stomach of most slugs: if the digestive tract of snails is unwound, the two positions are the same. Generally, in the families here studied, there are no internal ridges extending from the duct openings of the digestive gland to the intestine, as are found in *Oxychilus* (Rigby, 1963) or *Agriolimax* (Runham, 1975). The most important differentiations are found in succineids, where the ducts of the digestive gland are connected by a deep groove (Rigby, 1965), and *Gymnarion*, where two short and unequal typhlosoles extend to the beginning of the proximal intestine (Fig. 4). In most Stylommatophora the anterior duct of the digestive gland opens into the angle formed by the crop and the intestine either through a slit (Fig. 2) or through an oval or round aperture (*Gymnarion*, Fig. 4). The posterior duct forms an angle of about 90° with the anterior duct and opens through a round aperture into the posterior columellar side of the stomach.

In all Stylommatophora but a few carnivorous slugs, the intestine goes forward along the left side of the anterior digestive tract to turn around the aorta, clockwise when seen in dorsal view. When it opens from the right side of the stomach it has to pass under the crop or the oesophagus. From the aorta, the intestine has one bend backward before going forward again to the anus. The two bends of the intestine will be called the periaortic bend and the prerectal bend. In some slugs the body cavity is too short to house the two intestinal bends along the crop, and as a result the prerectal bend coils around the crop or even in the posterior part of the foot cavity,

behind the stomach (*Elisolimax*, Fig. 12; *Milax gracilis* [Leydig], Watson, 1930, pl. 2, fig.12). As demonstrated below, this is a secondary coiling, clearly not homologous in any way with the coil of the visceral mass in snails. Except for a few rectal ridges the intestine of helicarionids, succineids and athoracophorids does not show any internal morphological differentiation.

Torsion and hypertorsion of the stylommatophoran digestive tract

In embryos of stylommatophoran snails, torsion is about 90° counterclockwise in dorsal view, head anterior, previous to the development of a helicoidal visceral mass (*Helix*, Fol, 1880; *Achatina*, Ghose, 1963; Fretter, 1975). In the anterior digestive tract of most snails and slugs, this 90° torsion opens the oesophagus into the left anterior extremity of the crop, and results in the disposition of the crop floor on the right side of the foot cavity instead of the pedal side (for example, Fig. 8). Just after torsion of the embryo's visceral mass, the intestine opens dorsally from the left side of the stomach and goes directly to the left side of the aorta without having to cross the crop or oesophagus. In this stage the posterior duct of the digestive gland opens into the left side of the stomach and the anterior duct opens into the right side of the stomach (Ghose, 1963). In any adult snail with a coiled visceral mass, the intestine passes under the crop or oesophagus, describing a half circle around them before joining the aorta (Fig. 1 and 15; Orthurethura, Steenberg, 1925; also examined but not figured here in about one hundred species). Thus, the posterior part of the digestive tract of Stylommatophora has been hypertorted during ontogeny after torsion of the embryo. The degree of this hypertorsion may be determined: there is no hypertorsion when the intestine runs directly from the upper left side of the stomach to the left side of the aorta (observed only in embryos and in some slugs, as *Estria,* Fig. 10); there is a 360° hypertorsion when the intestine also issues from the upper left side of the stomach but describes a complete whorl around the right side of the crop before turning backward around the aorta (observed in some slugs, as *Elisolimax,* Fig. 12). In bulimoid snails with an elongated spire it seems that, at least for a few whorls, the degree of hypertorsion is somewhat a function of the number of whorls of the helicoid visceral mass occupied by the posterior digestive tract: 90° in *Succinea propinqua* Drouët for half a whorl occupied (Fig. 16); 120° in *Succinea putris* (L.) for a three-quarter whorl occupied (Fig. 15); 180° in *Euglandina carminensis* (Morelet) for one and a quarter whorls occupied. This hypertorsion is primarily the result of the presence of the posterior digestive tract in the helicoidal visceral mass. A helix is indeed the result of winding plus torsion: the winding corresponds to the projection of the spire on a plane perpendicular to its axis, and torsion is proportional to the height of the spire, which seems to be approximately the case for hypertorsion of high-spired snails. But this explanation of hypertorsion as primarily a mechanical result of the development of the visceral mass and posterior digestive tract into a high conical spire requires further discussion; in fact, shell shape varies in Stylommatophora from bulimoid and nearly parallel to foot length to helicoid or even flat, with shell axis perpendicular to foot length as in *Kalidos* (Fig. 1). Hypertorsion is at least 90° in all helicoid or planorboid snails that I have dissected, and is often more. The most common case is the opening of the intestine from the columellar lower side of the stomach (= lower right side), as in *Kalidos.* Furthermore, as shown in this paper, hypertorsion is preserved in most stages of shell reduction and even exaggerated in some slugs, whereas it is lost in others. We have no data about hypertorsion in the embryo of flat-shelled snails, but Fol (1880) described a 180° hypertorsion when the visceral mass of the embryo of *Limax* sinks down into the foot cavity. Hypertorsion may result from the development of an elongated helicoid visceral mass in a hypothetical bulimoid stylommatophoran ancestor and may be retained for other reasons in descendants having a different shell shape, but this is at the moment purely hypothetical.

The problem of variations in torsion of the digestive tract of Stylommatophora may be treated in three ways: as relationships of hypertorsion and shell shape, as variation found in the limacisation of flat-shelled snails, or as variation found in the limacisation of bulimoid snails. This paper analyses mainly the variations in the disposition of the digestive tract found in flat-shelled snails, through the example of the helicarionids which show various patterns. The variations found in the limacisation of bulimoid snails is analyzed through the example of the suc-

cineids; as far as I know from dissections in other families (oleacinids and acavids), it is representative of the general pattern found in the limacisation of bulimoid snails.

LIMACISATION OF HELICARIONIDS

Snails belonging to diverse groups have been used to understand how the digestive tract is modified in limacisation. Knowing the direction of evolutionary change, from snails to slugs, and applying the principle of parsimony, it is easy to arrange linearly the different steps observed in a number of series as little as possible. This does not imply at all monophyly of different steps of each series; it implies that in each series the ancestors of the most advanced slugs probably passed through stages in which they were similar to animals here considered representative of less advanced stages of the same evolutionary series. This means that evolution from snails to slugs is canalized. There are only a limited number of patterns, and when limacisation advances, the number of possible options for further steps is more and more limited.

The suprageneric groups of Asian and Pacific helicarionids used here were defined by Baker (1941) and revised by Solem (1966). The taxonomic basis for African groups is defined by Van Mol (1970) and Van Goethem (1977). The suprageneric groups are not necessarily used here at the same rank as these authors used, which changes nothing in the analysis of evolutionary trends. Here the name Helicarionidae includes the Ariophantinae, Durgellinae, Dyakiinae, Gymnarioninae, Girasiinae, Helicarioninae, Parmarioninae, Sesarinae, Trochozonitinae and Urocyclinae of various authors. As defined, the helicarionids occur from Tasmania to South Africa through the Indo-Australian Archipelago, Southeast Asia, India, Madagascar and the Ethiopian region. They are absent from the Middle East and Iran.

The first steps of limacisation

Helicarionids with a well-developed shell, up to six whorls (more in a few species), are found all over the group's range. No African or Asian helicarionid snail was examined here. They are probably similar to Madagascan and Melanesian species here examined, but it is not absurd to suppose from slugs described further that at least their oesophagus is longitudinally ribbed. Madagascan and New Caledonian helicarionids examined have no internal morphological differentiation of their digestive tract; it is possibly a secondary loss, but I have no argument to accept or reject this hypothesis.

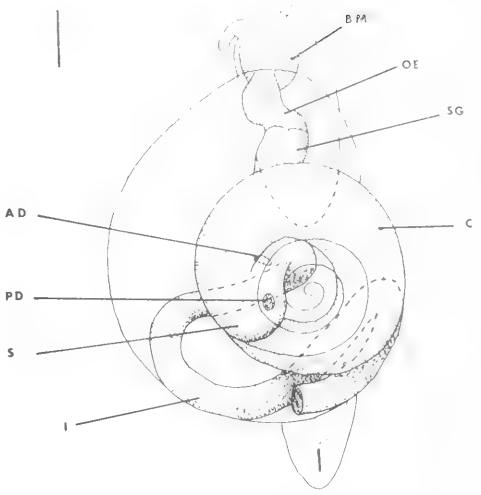

FIG. 1. *Kalidos oleatus* (Ancey), Madagascar. Rectum removed. Scale line 5 mm. AD, anterior duct of digestive gland; BM, buccal mass; C, crop; I, intestine; OE, oesophagus; PD, posterior duct of digestive gland; S, stomach; SG, salivary glands.

In a four-whorled *Kalidos oleatus* (Ancey) from Madagascar (Fig. 1), the lung occupies about one-third of the body whorl and the foot cavity is shorter than the tail. The oesophagus is longer than the crop into which it passes progressively at the level of the upper end of the lung. The intestine opens from the columellar upper end of the stomach, and passes under the oesophagus (hypertorsion) to reach the left side of the aorta. The two ducts of the digestive gland are almost circular in section and open into both sides of the entry of the intestine, forming an angle of nearly 180°. The anterior duct opens between the crop and the intestine, and the posterior duct opens into the columellar side of the stomach.

A New Caledonian two and three-quarter-whorled helicarionid has the same basic disposition, but the oesophagus, crop and lung are all shorter than in *Kalidos* (Fig. 2). As a result, the intestine passes under the crop. The transition from oesophagus to crop is more abrupt and the opening of the anterior

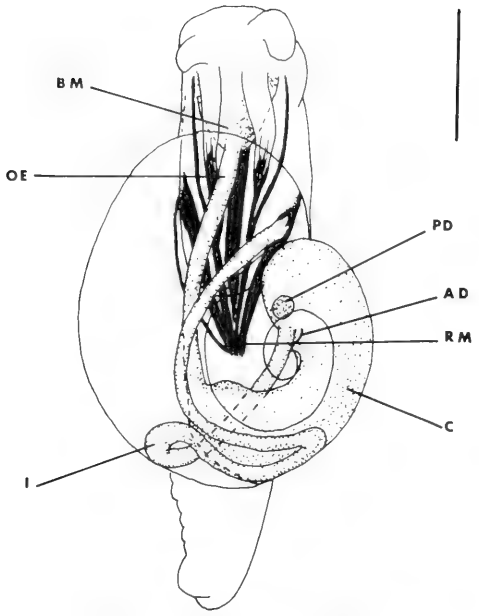

FIG. 2. Helicarionidae sp., New Caledonia. Scale line 2.5 mm. AD, anterior duct of digestive gland; BM, buccal mass; C, crop; I, intestine; OE, oesophagus; PD, posterior duct of digestive gland; RM, retractor muscles.

duct of the digestive gland is a transverse slit here, but these two characters probably do not reflect a general trend in the limacisation of helicarionids.

Semislugs

Semislugs are snails in which shell reduction has proceeded so far, and with such drastic shortening of the oesophagus, that the crop (when it is not separated from the stomach by a section of oesophagus) is at least partly contained in the foot cavity and the animal cannot retract inside the shell. However, the posterior edge of the foot cavity is lower and further forward than the most posterior part of the digestive tract, and the stomach is retained in the upper visceral cavity. In the limacisation of semislugs the crop and stomach are progressively uncoiled and become parallel to foot length, but may or may not retain hypertorsion.

Plesiomorphic hypertorted semislugs. The reduction in number of visceral mass whorls results in the reduction of the foot and visceral cavities. The lung is reduced and the kidney either is shortened or becomes transverse and folded onto itself, as described by Solem (1966), Van Mol (1970) and Van Goethem (1970) in helicarionids. These modifications of the pallial complex are not sufficient to preserve the same ratio of internal volume/body size; the digestive system has to be reduced, and/or the foot cavity enlarged. Theoretically, all digestive tract parts could be reduced and retain the same proportions. In fact, at least in helicarionid semislugs, the stomach and crop retain approximately the same size, whereas oesophagus length is largely reduced and intestine length less reduced. As shown by the Madagascan *Malagarion* (Fig. 3) and the West African *Gymnarion* (Fig. 4), the crop partly sinks into

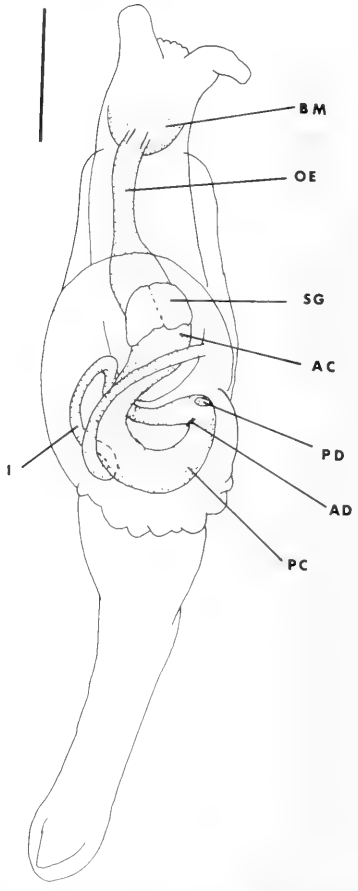

FIG. 3. *Malagarion paenelimax,* Madagascar. Scale line 5 mm. AC, anterior crop; AD, anterior duct of digestive gland; BM, buccal mass; I, intestine; OE, oesophagus; PC, posterior crop; PD, posterior duct of digestive gland; SG, salivary glands.

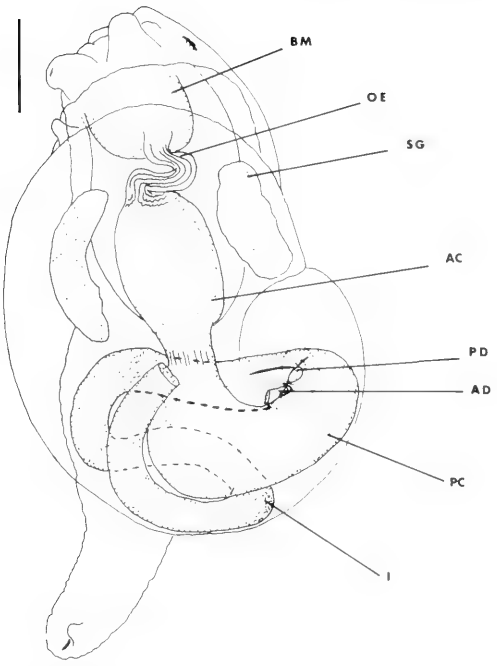

FIG. 4. *Gymnarion sowerbyanus,* Ivory Coast. Rectum removed. Scale line 5 mm. AC, anterior crop; AD, anterior duct of digestive gland; BM, buccal mass; I, intestine; OE, oesophagus; PC, posterior crop; PD, posterior duct of digestive gland; SG, salivary glands.

the foot cavity, which in this stage keeps the same relative length as in snails. When the passage from foot cavity to visceral cavity is necessarily reduced, because of the presence of the lung, if the foot cavity is not enlarged, the crop is divided into two inflated chambers separated by a constriction: the anterior crop, contained in the foot cavity, and the posterior crop, contained in the visceral cavity. The visceral part of the digestive tract retains hypertorsion and the intestine passes under the constriction of the crop (the figure of the digestive tract of *Malagarion* in Tillier, 1979, is erroneous). The digestive tract of *Malagarion* has no internal ridges. *Gymnarion* has oesophagal ridges, a few longitudinal ridges in the constriction of the crop, plus a few small ridges in the stomach region (two thin longitudinally unequal ridges from the openings of the digestive gland, one small transverse ridge).

The constriction between foot cavity and visceral cavity, which differentiates an anterior and a posterior crop, is clearly related to the anterior position of the visceral cavity edge in the groups studied here. If the visceral mass is further back, as in the family Vitrinidae, the passage from the foot cavity to the visceral cavity is wider and the crop keeps the same diameter from the end of the oesophagus to the stomach (Tillier, unpublished).

Apomorphic semislugs retaining hypertorsion. When the shell and visceral cavity are reduced more than in *Malagarion* and *Gymnarion*, the part of the digestive tract anterior to the stomach is entirely uncoiled and the whole crop is parallel to body length. Such a disposition is shown by the African *Mesafricarion maculifer* Pilsbry (Fig. 5; slightly contracted). In this species the hermaphrodite gland is retained on the right side of the visceral mass. The two lobes of the digestive gland and their ducts occupy, as a result, a position different from the one found in *Gymnarion*: the anterior duct is in a lower left and posterior position, whereas the posterior

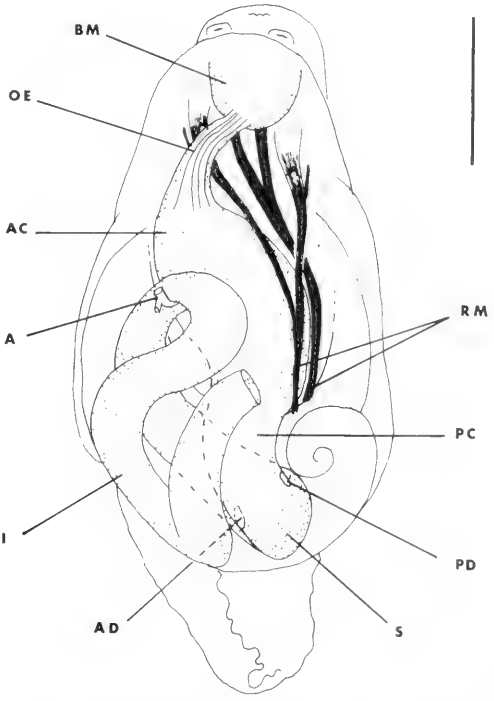

FIG. 5. *Mesafricarion maculifer,* Zaïre. Rectum removed. Scale line 5 mm. A, aorta; AC, anterior crop; AD, anterior duct of digestive gland; BM, buccal mass; I, intestine; OE, oesophagus; PC, posterior crop; PD, posterior duct of digestive gland; RM, retractor muscles; S, stomach.

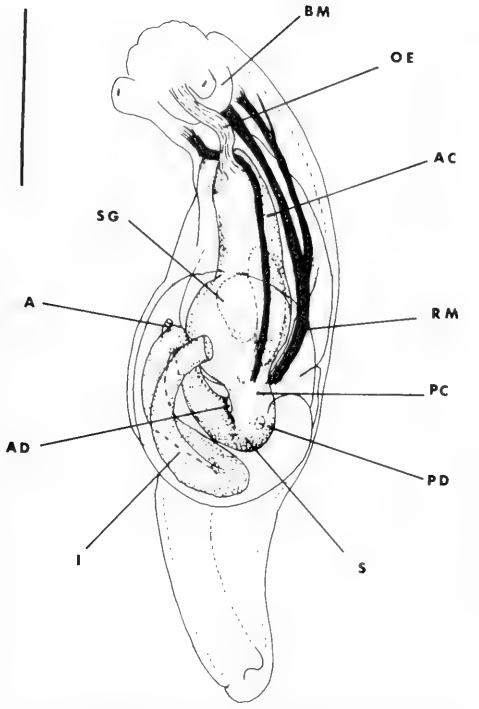

FIG. 6. *Parmarion* sp., Cambodia. Rectum removed. Scale line 2.5 mm. A, aorta; AC, anterior crop; AD, anterior duct of digestive gland; BM, buccal mass; I, intestine; OE, oesophagus; PC, posterior crop; PD, posterior duct of digestive gland; RM, retractor muscles; S, stomach; SG, salivary glands.

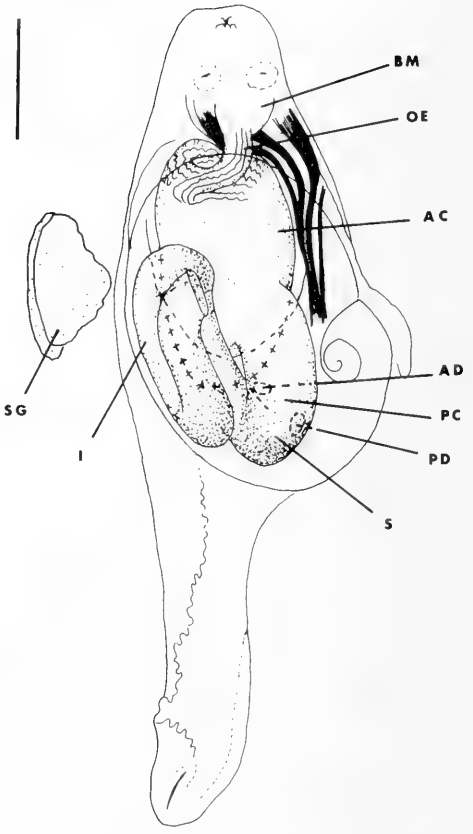

FIG. 7. *Granularion lamottei*, Guinea. Rectum removed. Scale line 5 mm. AC, anterior crop; AD, anterior duct of digestive gland; BM, buccal mass; I, intestine; OE, oesophagus; PC, posterior crop; PD, posterior duct of digestive gland; S, stomach; SG, salivary glands.

duct is in the position where the anterior duct would be expected. The genital apparatus is entirely contained in the lower and right part of the foot cavity, the hermaphroditic gland excepted.

Apomorphic semislugs partly losing hypertorsion. In some advanced semislugs, such as the Asiatic *Parmarion* (Fig. 6) or the West African *Granularion* (Fig. 7), the posterior part of the crop and stomach are not only parallel to the body axis, but also partly detorted when compared with the *Gymnarion-Malagarion* stage. The posterior crop is no longer differentiated from the stomach and proximal intestine in diameter, and the intestine now opens to the left and forward from a stomach. As a result of this 90° detorsion clockwise, the posterior duct of the digestive gland opens upward into the stomach, whereas it would open downward without detorsion. It still forms an angle of about 90° with the anterior duct. The anterior crop is more developed than in former stages of limacisation and is entirely contained in the foot cavity. In *Parmarion*, only a few thin oesophageal ridges are found, whereas in *Granularion* these ridges are thicker and extend into the anterior extremity of the crop, where they are wrinkled. In *Parmarion*, the foot cavity is longer than the tail. In *Granularion*, it is shorter and its posterior extremity is further back than its posterior upper edge: the tail is partly hollow.

It cannot be proven that partial detorsion did not happen sooner in limacisation than this stage, given our present stage of knowledge. It is improbable, but not impossible.

Slugs

A semislug evolves into a slug when the stomach sinks into the foot cavity, lower than the posterior edge of the foot cavity. When this process occurs, this edge of the foot cavity may be in front or behind the posterior end of the digestive tract and visceral cavity. As an advanced semislug may have retained or lost hypertorsion of the digestive tract, four situations are possible, depending on which type of semislug is ancestral: 1) a partly detorted semislug having the posterior edge of the foot cavity in front of the stomach; 2) behind it; 3) a hypertorted semislug having the posterior edge of the foot cavity in front of the stomach; 4) behind it. When the posterior edge of the foot cavity is in front of the stomach, the tail becomes progressively hollow posteriorly and the digestive tract has to turn around to go down into the enlarged foot cavity. When the posterior edge of the foot cavity is behind the stomach, the foot becomes more hollow ventrally and the digestive tract may sink directly into it without any change in its disposition.

Slugs originating from partly detorted semislugs.

Group I: Posterior upper edge of foot cavity anterior to stomach. This process may be illustrated by the *Estria-Rhopalogonium* group, West African taxa considered monophyletic by Van Goethem (1977). By its general as well as genital anatomy, *Granularion* is probably a member of this group less advanced in limacisation. As described above, in this genus only the crop is in the foot cavity that enters a little into the tail (Fig. 7). *Estria?* sp. A (Van Goethem, 1977) may be representative of the next step in limacisation from a similar ancestor (Fig. 8). The foot cavity is longer than in *Granularion*, although its upper edge occupies nearly the same position. The stomach sinks into the foot and forms a bend in a plane perpendicular to foot sole and foot length. The intestine is entirely included in the foot cavity on the left side of the crop. In *Tresia parva* Van Goethem (Fig. 9), the foot cavity extends backward to the middle of the tail; the disposition of the digestive tract is similar but here the bend formed by the stomach can spread parallel to foot sole. The loss of hypertorsion is achieved in the stomach region where the intestine opens from the left side of the stomach. The process is completed in *Estria* (Fig. 10, redrawn from Poirier, 1888), which has a still longer foot cavity where the oesophagus and crop are straight again, as in the ancestor apomorphic semislug, and parallel to foot length. The loss of hypertorsion appears definitely retained.

In *Granularion*, the oesophagus has straight folds, prolonged inside the ventral side of the crop by a few wrinkled ridges; the sides and upper surface of the crop have internal, thin, scarce transverse ridges. *Tresia* has only oesophageal folds. No *Estria* could be directly observed.

Group II: Posterior upper edge of foot cavity shifted backward. The final stage of this process is illustrated by the Indian *Mariaella* (Fig. 11). The transformation from a disposition similar to the one found in *Parmarion* (Fig. 6)

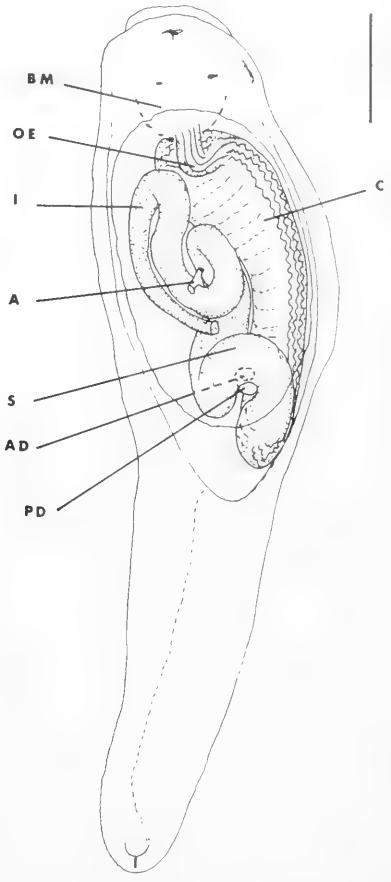

FIG. 8. *Estria?* sp. A (Van Goethem, 1977), Ivory Coast. Rectum removed. Scale line 5 mm. A, aorta; AD, anterior duct of digestive gland; BM, buccal mass; C, crop; I, intestine; OE, oesophagus; PD, posterior duct of digestive gland; S, stomach.

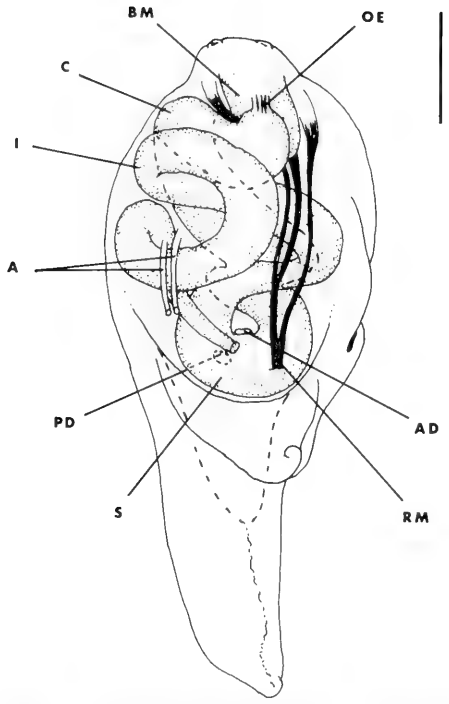

FIG. 9. *Tresia parva* Van Goethem, paratype, Guinea. Rectum removed. Scale line 2.5 mm. A, aorta; AD, anterior duct of digestive gland; BM, buccal mass; C, crop; I, intestine; OE, oesophagus; PD, posterior duct of digestive gland; RM, rectractor muscles; S, stomach.

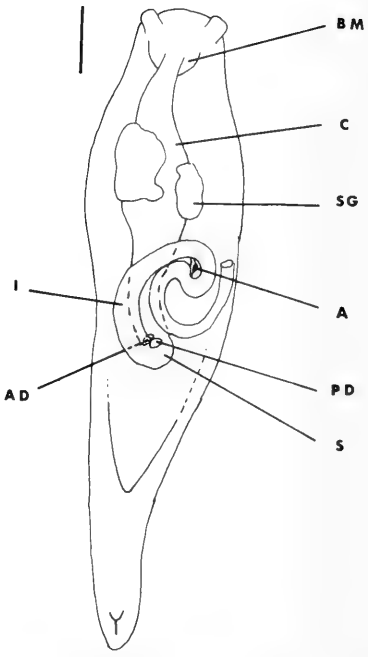

FIG. 10. *Estria alluaudi,* Ivory Coast, reproduced from Poirier (1888). Scale line 5 mm. A, aorta; AD, anterior duct of digestive gland; BM, buccal mass; C, crop; I, intestine; PD, posterior duct of digestive gland; S, stomach; SG, salivary glands.

is a simple vertical translation ventrally, allowed by the position of the upper posterior edge of the foot cavity further back. Here is a large distance between the two ducts of the digestive gland. The oesophagus has a few longitudinal ridges; the crop has a few transverse ridges. The crop forms a kind of rostrum in front and beneath the opening of the oesophagus, an arrangement that is also found more or less developed in *Gymnarion, Parmarion, Granularion* and *Estria?* sp.A (Figs. 4, 6, 7, 8). The intestine has a few longitudinal short ridges in the beginning of the periaortic bend. In *Mariaella* the lung is prolonged downward and backward by air sacs; the frontal air sac separates the previous foot cavity from the previous visceral cavity.

Austenia doisutepensis Solem from Cambodia possibly illustrates a process of limacisation by loss of hypertorsion with the foot cavity edge in an intermediate position, but the stomach is not located in Solem's figure (1966) and it is difficult to determine.

Slugs originating from hypertorted semislugs.

Group III: Posterior edge of foot cavity anterior to stomach. Slugs possibly illustrating the steps of limacisation from such a semislug were not observed, which does not prove that it did not or does not occur. In such a process, which would start from a semislug similar to *Mesafricarion* (Fig. 5) but having the visceral hump further in front, the proximal intestine should go down first into the right part of the foot cavity, followed by the stomach and finally the posterior crop. Intermediate positions do not seem very functional, mainly because the proximal intestine would have to occupy the space normally filled by the genital apparatus. However, hypertorted slugs having the lung in the anterior part of the body, as *Arion*, could have evolved through such a process; the necessity for housing the proximal intestine on the right side of the crop in the intermediate steps of limacisation would ex-

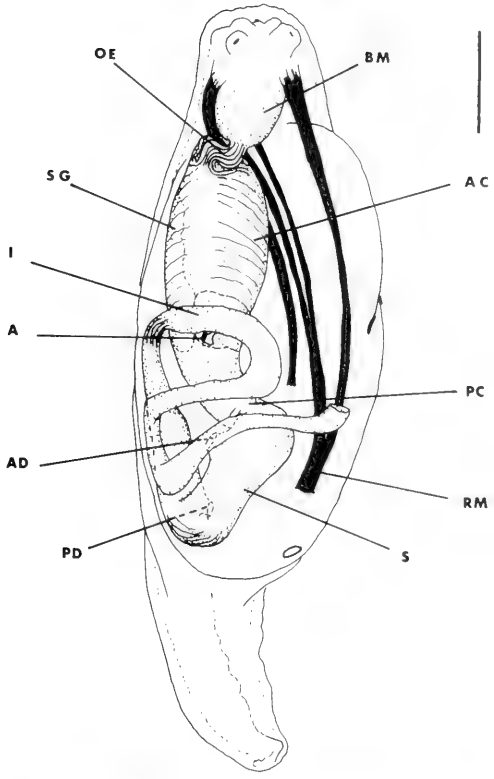

FIG. 11. *Mariaella dussumieri*, probably southern India. Rectum removed. Scale line 5 mm. A, aorta; AC, anterior crop; AD, anterior duct of digestive gland; BM, buccal mass; I, intestine; OE, oesophagus; PC, posterior crop; PD, posterior duct of digestive gland; RM, rectractor muscles; S, stomach; SG, salivary glands.

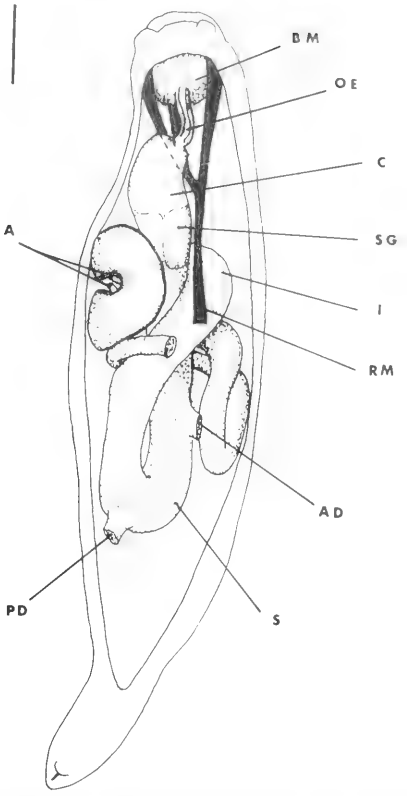

FIG. 12. *Elisolimax madagascariensis,* Madagascar. Rectum removed. Scale line 5 mm. A, aorta; AD, anterior duct of digestive gland; BM, buccal mass; C, crop; I, intestine; OE, oesophagus; PD, posterior duct of digestive gland; RM, retractor muscles; S, stomach; SG, salivary glands.

plain why the spermoviduct is on the left side in *Arion* (general anatomy of *Arion* depicted by Van Mol, 1962, digestive tract slightly detorted for drawing).

Group IV: Posterior upper edge of foot cavity behind stomach. In such a case the hypertorted digestive tract, similar to the one of *Mesafricarion* (Fig. 5), sinks into the foot cavity, enlarged ventrally. The intermediate steps of this process have not yet been observed in helicarionids, but *Parmacella* illustrates this process in another family (Tillier, unpublished).

Exaggeration of hypertorsion in urocycline slugs. In *Elisolimax* the intestine opens from the upper left end of the stomach and makes a complete coil around the crop before turning around the aorta (Fig. 12). This is half a whorl more than in slugs directly derived from a hypertorted semislug similar to *Mesafricarion*. Van Goethem (1977) believes that the Urocyclini are related to *Mesafricarion* on the basis of their genital characters. If he is right and the Urocyclini ancestor was similar to *Mesafricarion*, this implies that exaggeration of hypertorsion occurred either during the intermediate steps, or after limacisation. Exaggerated hypertorsion may be related to the maintenance of the whole genital apparatus on the right side of the digestive tract. No observation makes these hypotheses unlikely. In particular, the anterior duct of the digestive gland of *Elisolimax* is in the position expected if the digestive tract of *Mesafricarion* is hypertorted 180° (Figs. 5, 12), and the exaggeration of hypertorsion is obviously easier to obtain from a hypertorted semislug than from a detorted one. *Atoxon* (Fig. 13) is

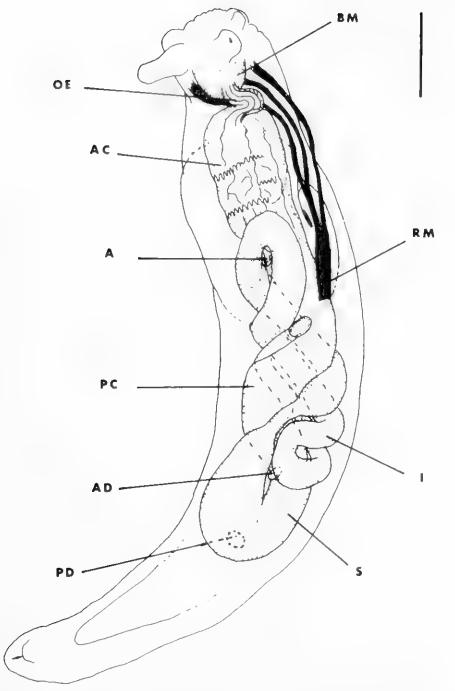

FIG. 13. *Atoxon pallens*, Zaïre. Rectum removed. Scale line 5 mm. A, aorta; AC, anterior crop; AD, anterior duct of digestive gland; BM, buccal mass; I, intestine; OE, oesophagus; PC, posterior crop; PD, posterior duct of digestive gland; S, stomach.

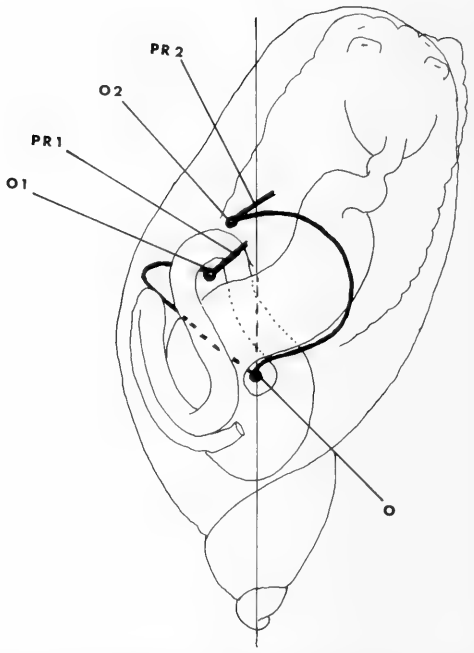

FIG. 14. Possible evolutionary migration of insertion of penial retractor along inner wall of body cavity, from hypothetical columellar origin "0." 01, position found in succineids; 02, position found in other Aulacopoda; PR1 and PR2: corresponding position of penial retractor. Outline of *Succinea putris* (Fig. 15). Rectum removed.

modified still more than *Elisolimax* when compared with *Mesafricarion*. The anterior crop is differentiated by internal ridges, and the prerectal intestinal bend is longer, which puts the anterior duct of the digestive gland farther to the left.

LIMACISATION OF SUCCINEIDS

In succineids which are plesiomorphic in limacisation, e.g. *Succinea putris* (Fig. 15), the shell is bulimoid and only slightly oblique with relation to foot length. The shell has little more than three whorls in *Succinea putris* and not much more in any succineid snail. When compared to stylommatophoran standards for whorl number, even the most plesiomorphic succineids are advanced in shell reduction. The absence of succineid species or genera having a more developed visceral mass, intermediate between the stage observed in *Succinea* and the stage observed in most snails (at least five whorls) probably explains why succineids were considered as a distinct group for such a long time. The relative compaction of the succineid oviduct when compared with the spermoviduct of most Stylommatophora, and probably the transverse position of the kidney, may be the result of the first steps in limacisation. The compaction of the spermoviduct is observed in bulimulid semislugs (Van Mol, 1971), and the kidney is transverse in Asian advanced helicarionids (although folded and retaining an U-shaped ureter; Solem, 1966). In the oleacinids, another family of bulimoid snails with trends in shell reduction, the kidney also has odd arrangements: the kidney is wider than long in the Streptostylini, and the primary ureter is independent of the rectal side of the kidney in the Euglandini. Possibly because all intermediates between an eight-whorled shell and a semislug occur, these original trends were never used to define a suborder, as in the case of succineids.

Even the most plesiomorphic succineids differ basically from all families studied here in

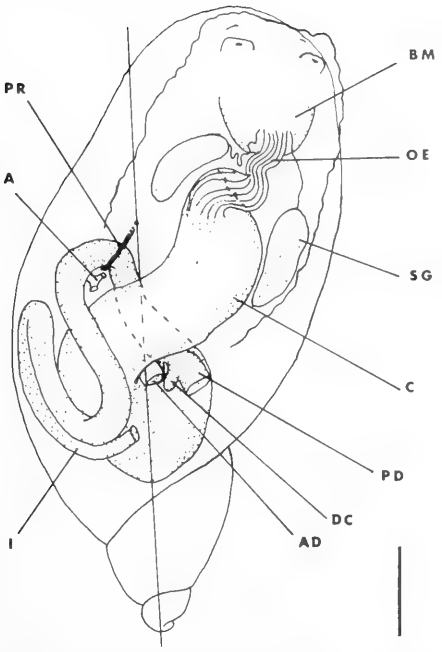

FIG. 15. *Succinea putris*, France. Rectum removed. Scale line 2.5 mm. A, aorta; AD, anterior duct of digestive gland; BM, buccal mass; C, crop; DC, digestive caecum; I, intestine; OE, oesophagus; PD, posterior duct of digestive gland; PR, penial retractor; SG, salivary glands.

the insertion position of their penial retractor, which begins beside the origin of the aorta, inside the periaortic bend of the intestine (01, Fig. 14 and Tillier, 1981, fig. 2). In all Aulacopoda which I have examined, except in a few endodontids where the penial retractor is a branch of the columellar retractor, it starts from the lung floor, lung border or body wall outside the periaortic bend of the intestine (02, Fig. 14). If we consider the penial retractors of all Stylommatophora as homologous, the position of its insertion implies that from a probable columellar origin it shifted to the left in succineids, whereas it migrated to the right and then to the left around the crop in all other Aulacopoda. This purely theoretical migration is shown in Fig. 14. From a columellar origin 0, the insertion of the penial retractor may have migrated along the palatal wall of the visceral cavity to 01 in succineids, the retractor passing under the crop and above the proximal intestine all along its migration. In other Aulacopoda, it may have migrated from the same origin 0 along the floor of the lung to 02, the retractor passing above the crop all along its migration. In Fig. 14 the outline of *Succinea putris* is used for clarity although this process, if it ever occurred, probably did so in a stage in which the shell and visceral mass were much more developed than in *Succinea*.

In *Succinea putris* (Fig. 15), the oesophagus is ribbed, short and opens into the left anterior end of the crop (torsional twist of Rigby, 1965). The crop is long and makes nearly one whorl in the visceral mass before ending, without any morphological discontinuity, in the stomach. The hypertorsion of the stomach and proximal intestine is about 120°. The anterior duct of the digestive gland opens backward into the angle formed by the crop and proximal intestine. The posterior duct opens into the columellar side of the stomach and forms an angle of about 90° with the anterior duct. The posterior duct has a caecum in which the groove that joins the openings of the ducts internally ends.

In the Guyanese *Succinea propinqua*, the visceral mass has about one whorl less than in *Succinea putris* (Fig. 16). The oesophagus is shorter in the former and has only very thin longitudinal ridges. Hypertorsion and the oesophageal torsional twist are partly lost. As a result, the posterior duct of the digestive gland opens upward and opposite the columellar side of the stomach. Its caecum opens at the junction of the duct and intestine.

In *Hyalimax perlucidus* (Quoy & Gaimard) from the Mascarenes the reduced shell and visceral mass are completely covered by the mantle (Fig. 17). The foot cavity is not enlarged as compared with the foot cavity of *Succinea*, whereas the upper visceral cavity is greatly reduced. This situation results in great reduction of all parts of the digestive tract, a unique feature. Furthermore, the digestive tract has not only large oesophaageal ridges, but also thin crop ridges that run along the right inner side of the crop. Because of detorsion, these ridges that run to the posterior duct of the digestive gland are dorsal, whereas in a similar position they would be ventral in a torted slug. The crop ridges and also sacculations of the intestine, observed only in *Hyalimax* among succineids examined, may be related to the general relative reduction in size of the digestive tract as they increase the internal digestive surface. The arrangement of the digestive tract is otherwise about the same as in *Succinea propinqua*. Detorsion is, however, a little more

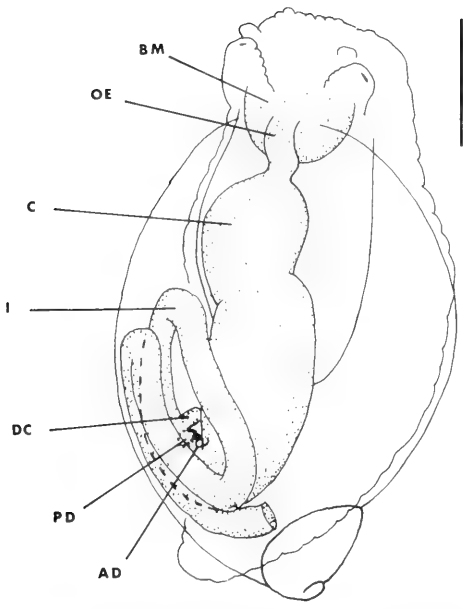

FIG. 16. *Succinea propinqua*, French Guyana. Rectum removed. Scale line 2.5 mm. AD, anterior duct of digestive gland; BM, buccal mass; C, crop; DC, digestive caecum; I, intestine; OE, oesophagus; PD, posterior duct of digesive gland.

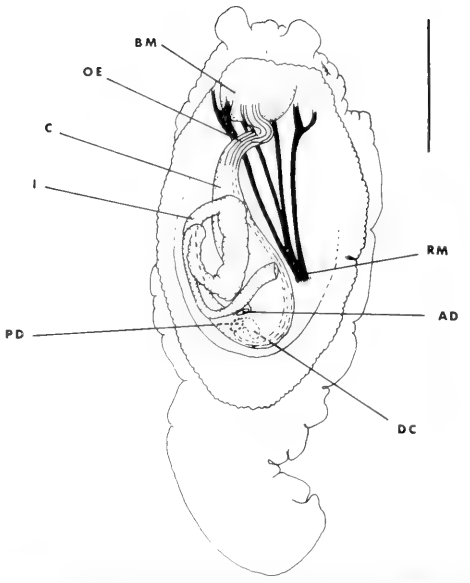

FIG. 17. *Hyalimax perlucidus*, La Réunion. Rectum removed. Scale line 5 mm. AD, anterior duct of digestive gland; BM, buccal mass; C, crop; DC, digestive caecum; I, intestine; OE, oesophagus; PD, posterior duct of digestive gland; RM, retractor muscles.

accentuated here, and as a result the posterior duct of the digestive gland and its caecum open further underneath the stomach than in *Succinea propinqua* (compare Figs. 16 and 17).

In South American *Omalonyx matheroni* (Potiez & Michaud), the foot cavity is largely expanded backward when compared with the foot cavity of *Succinea* (Fig. 18). The proportions of the different parts of the digestive tract are still about the same, and its relative size is much larger than in *Hyalimax*. The digestive tract is still more detorted than in *Hyalimax*. The intestine is in an upper position instead of occupying the left side of the body cavity, and the posterior duct of the digestive gland and its caecum open into the posterior lower side of the stomach.

From the *Succinea propinqua* to the *Omalonyx* stage of limacisation the torsional twist of the oesophagus and anterior crop is lost. The ventral side of the crop, which is the right side in *Succinea putris* as in most Stylommatophora, because of torsion, is the left side in succineid semislugs and slugs because of detorsion plus loss of hypertorsion. This is why the crop ridges of *Hyalimax* are dorsal, as shown by their leading to the posterior duct

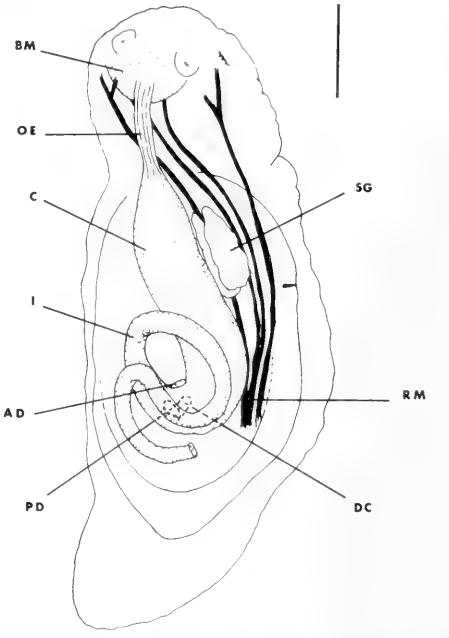

FIG. 18. *Omalonyx matheroni*, French Guyana. Rectum removed. Scale line 5 mm. AD, anterior duct of digestive gland; BM, buccal mass; C, crop; DC, digestive caecum; I, intestine; OE, oesophagus; PD, posterior duct of digestive gland; RM, retractor muscles; SG, salivary glands.

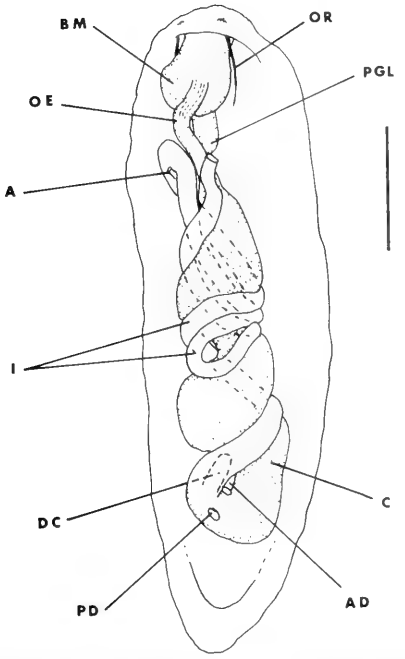

FIG. 19. Athoracophoridae n. sp., New Caledonia. Rectum removed. Scale line 5 mm. A, aorta; AD, anterior duct of digestive gland; BM, buccal mass; C, crop; DC, digestive caecum; I, intestine; OE, oesophagus; OR, ocular retractor; PD, posterior duct of digestive gland; PGL, pedal gland.

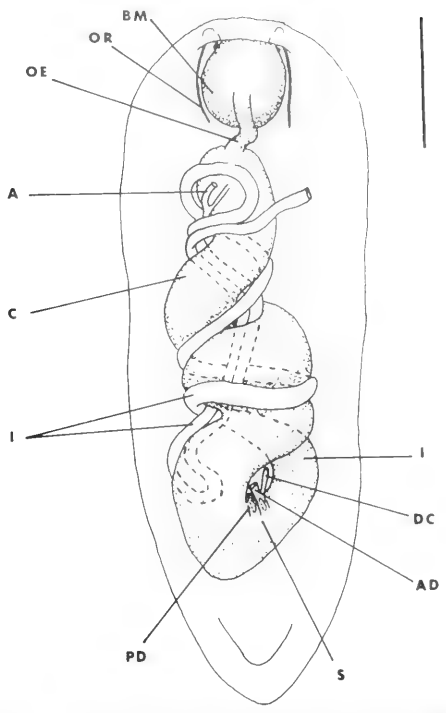

FIG. 20. *Aneitea simrothi* Grimpe & Hoffmann, New Caledonia. Rectum removed. Scale line 5 mm. A, aorta; AD, anterior duct of digestive gland; BM, buccal mass; C, crop; DC, digestive caecum; I, intestine; OE, oesophagus; OR, ocular retractor; PD, posterior duct of digestive gland; S, stomach.

of the digestive gland, which is basically dorsal.

It may be questioned whether detorsion is an obligatory consequence of limacisation when the shell is bulimoid and nearly parallel to foot length. It is quite possible, as far as hypertorsion is a result of the helicoid growth of the visceral mass during ontogeny (as in *Achatina*) and not of rotation of the embryonic visceral mass (as in *Limax*), as discussed above. In such cases as *Achatina*, the loss of the helicoid spire should imply detorsion, as observed in succineids. The complete detorsion of the digestive tract of *Testacella* shown by Lacaze-Duthiers' figure (1887, for example pl. 24, fig. 47) could give an additional indication of such a relationship as far as Watson's hypothesis (1915) of the oleacinid (Streptostylini) origin of testacellids is verified.

COMPARATIVE ANATOMY OF ATHORACOPHORIDS

Burton (1980) concluded from his comparative study of Australian, New Zealand and Subantarctic athoracophorids that northern species, belonging to the genera *Aneitea, Aneitella* and *Triboniophorus*, are less advanced than southern species. Two northern (and thus probably plesiomorphic) species are used here for comparison with other families: *Aneitea simrothi* Grimpe & Hoffmann from New Caledonia (Fig. 20) and a new species from New Caledonia, considered by G. M. Barker as representative of an undescribed genus (unpublished), which will be referred to as Athoracophoridae n. sp. (Figs. 19 and 21).

In Athoracophoridae n. sp. the short, slightly ribbed oesophagus passes gradually into a very long and very large crop (Fig. 19). The stomach region is very close to the end of the tail. The homology of the two ducts of the digestive gland may be established from the disposition of the corresponding lobes of the gland. The posterior lobe is behind the stomach, not involved in the torsion of the digestive tract, and opens into the posterior upper side of the stomach. The anterior lobe

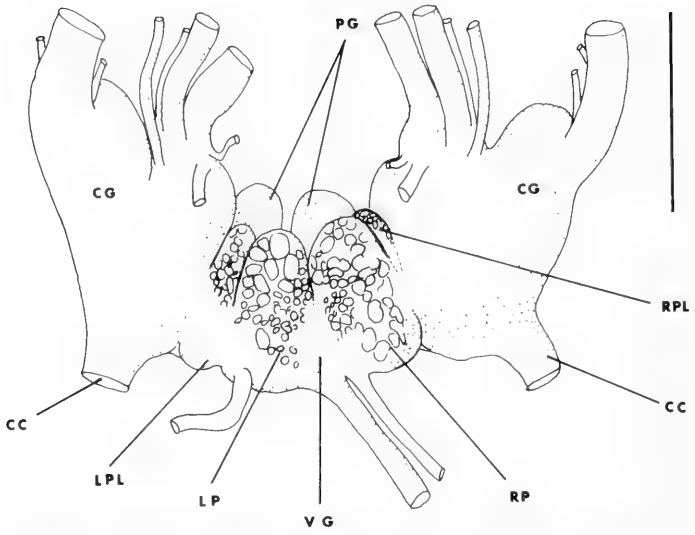

FIG. 21. Central nervous system of Athoracophoridae n. sp., cerebroid commissure cut and cerebroid ganglia drawn aside; dorsal view. Scale line 1 mm. CC, cerebroid commissure; CG, cerebroid ganglia; LP, left parietal ganglion; LPL, left pleural ganglion; PG, pedal ganglia; RP, right parietal ganglion; RPL, right pleural ganglion; VG, region corresponding to visceral ganglion.

is involved in the torsion of the digestive tract and coiled around the crop. It opens into the angle formed by the crop and intestine. Just distal to the anterior duct is a digestive caecum opening directly into the proximal intestine. The intestine opens from the upper left side of the stomach, as in urocycline slugs (compare Figs. 12 and 19), but makes two complete whorls around the crop before reaching the aorta, instead of one, as in the Urocyclini. The digestive tract of Athoracophoridae n. sp. is thus hypertorted one whorl when compared with the digestive tract of the Urocyclini, and one-and-a-half whorls when compared with the digestive tract of most Stylommatophora. The intestine is reduced in diameter when compared with the crop, and neither show any internal morphological differentiation. The digestive tract of *Aneitea simrothi* exhibits the same general features (Fig. 20), but here the intestine opens from the right lower side of the stomach and makes two and a half whorls around the crop before reaching the aorta. The digestive tract of *Aneitea simrothi* is thus hypertorted half a whorl more than in Athoracophoridae n. sp., and two whorls more than in most Stylommatophora. In *Aneitea simrothi* the prerectal bend of the intestine is so long that it passes between the crop and the proximal intestine, which is not the case in any other observed Stylommatophora, but this is clearly a consequence of lengthening plus hypertorsion. The two ducts of the digestive gland are contiguous in the angle formed by the crop and intestine, but it is still posible to establish their homology from the position of the lobes of the digestive gland. The posterior duct is on the pharyngeal and lower side of the stomach, whereas the anterior duct is on the intestinal and upper side, and the digestive caecum is here an appendix of the anterior duct, not opening directly into the intestine (it may be noticed that the anterior duct is in a posterior position with regard to the posterior duct). As far as may be seen from published figures of the digestive tract of the athoracophorids, the arrangement found in Athoracophoridae n. sp. is found only in *Aneitella* and *Aneitea simrothi* represents the general case (Plate, 1898; Glamann, 1903; Oberzeller, 1970; Burton, 1980). The occurrence of two steps in hypertorsion in the Athoracophoridae suggests that other steps have occurred, since the exaggeration of hypertorsion occurs in some other slugs such as the Urocyclini. The extreme exaggeration found in athoracophorids may thus be related to their being extremely advanced slugs, but does not prove at all that they have an unusual origin.

The pallial complex of athoracophorids has been accurately described by Plate (1898) and Glamann (1903); Delhaye & Bouillon

(1972) added more histological data. In the group considered by Burton (1980) as plesiomorphic, the general relative disposition of organs is similar to what is found in stylommatophoran slugs: kidney basically in the posterior left part of the lung cavity, heart in front of the kidney, pneumostome opening together with the rectum and ureter on the right side of the lung cavity. Differences from the general slug pattern are: lung cavity completely filled by a tridimensional net of connective tissue, including venous system and delimiting a system of air lacunae, called a tracheal lung by Plate (1898); ureter with appendices and circumvolutions, entirely deprived from the internal transverse lamellae which are supposed to characterize the primary ureter of sigmurethrous snails (Delhaye & Bouillon, 1972); ureter reaching the pneumostome along the front edge of the lung cavity, instead of along the back edge. Delhaye & Bouillon (1972) define the heterurethrous kidney as being without a sphincter surrounding its opening into the ureter, and say that their observations justify grouping athoracophorids with true Heterurethra, but do not describe the absence of an ureteric sphincter in the kidney of *Triboniophorus graeffei* Humbert. On the other hand, such a sphincter is clearly visible on Glamann's figure 10 (1903, pl. 30), representing a section of the pallial complex of *Aneitella virgata* passing through the kidney opening. It will thus be considered here that the kidney opening of athoracophorids has a sphincter, as found in at least some Sigmurethra. The cavities considered since Plate and Glamann as being a pneumostome (Athemgang) and a lung cavity (Athemhöle) are, from their own figures, the same structure: they are lined by an epithelium with cubic cells, sometimes glandular cells, and surrounded by muscular and conjunctive tissues. In all Stylommatophora, these are characters of the pneumostome, and I thus consider that what is called the lung cavity of athoracophorids is in fact a ramified pneumostome. The homologous part of the lung cavity of other Stylommatophora must be delimited by a wall without cubic cells, including a venous system: this is the "tracheal" lung of Plate. In fact, such a structure is not so uncommon among stylommatophoran semislugs and slugs, but is usually overlooked because it does not entirely fill the lung cavity. For example, Fischer & Crosse (1872: 193) mention the alveolar structure of the lung roof in *Parmacella*, *Xanthonyx* and *Pellicula*. This structure is visible in figures of Simroth & Hoffmann (1908–1928, pl. 23, fig. 4) and Van Mol (1971) showing, respectively, the lungs of *Parmacella* and *Peltella*. A section is shown by Simroth & Hoffmann (1908–1928, fig. 147) in *Anadenus*. In *Parmacella* and probably in other genera cited above, it is formed by expansions of the lung wall, including the venous system, into a net that increases the respiratory surface by forming an alveolar system (Tillier, 1982). If such a system is expanded to fill the whole lung cavity, it will form a cavity similar to the lung of athoracophorids. The lung of athoracophorids must thus be called alveolar because it is considered as formed by expansions of the lung wall forming alveoli, and not by invaginations of the epidermis forming trachea; only the athoracophorid pneumostome may be called tracheal. In *Parmacella*, the structure which has been called the "*glandula aciniformis*" by Wiktor & Likharev (1980) is in fact built from a complication of ureteric diverticula, venous system and air lacunae. Furthermore, the portion of the ureter from its proximal orifice to the back edge of the lung cavity, between the kidney and the alveoli, lacks internal lamellae, whereas such lamellae occur along the back edge of the lung cavity. The part of the ureter which is in the position of a primary ureter does not have the characteristic features of a primary ureter, whereas such a structure is found in the part which is in the position of a secondary ureter. This suggests that the formation of ureteric diverticula is related to the development of an alveolar lung and that either the absence of ureteric lamellae is not a primary feature of Sigmurethra, or these lamellae may be lost. Thus, these features have no primary systematic value. The position of the excretory duct in front of the lung cavity of athoracophorids is more difficult to explain without additional comparative data. It could be primarily an ureteric diverticulum, opening outside secondarily, or be homologous of a secondary ureter which could have migrated forward along the roof of the lung cavity (a movement equivalent to rotation around the axis of the pneumostome).

The reproductive system of athoracophorids is well known (Burton, 1978, 1980) and provides little data useful for between-families comparison. However, it is important to note that the penial retractor is inserted, as usual

among aulacopods, outside of the periaortic bend of the intestine, on the left upper side of the foot cavity.

The central nervous system may be important in phylogeny (Bargmann, 1930; Bishop, 1978). In most athoracophorids the suboesophageal ganglia are fused in a chain where only two large symmetrical masses can generally be distinguished (Plate, 1898; Oberzeller, 1970; Burton, 1962, 1980). In Athoracophoridae n. sp. the fusion is less complete and the outline of four suboesophageal ganglionic masses can be observed (Fig. 21). The left pleural ganglion is quite visible, whereas the right pleural is hidden under the right parietal ganglion; the right parietal ganglion is more voluminous than the left and the visceral ganglion is fused with both parietal ganglia. The nervous trunk issuing from the region equivalent to the visceral ganglion is closer to the nerve coming from the right parietal than to the nerve coming from the left parietal ganglion. This suggests that the fusion of the visceral ganglion is more achieved with the right parietal ganglion, and thus anterior to the fusion with the left parietal ganglion.

The pedal gland of athoracophorids lies free in the foot cavity (Fig. 19, PGL). This character was considered by Watson (1915, 1930) as having phylogenetic importance.

RELATIONSHIPS OF SUCCINEIDS, ATHORACOPHORIDS AND OTHER AULACOPODS

Since Mörch (1865) noticed that both athoracophorids and succineids have an elasmognathous jaw, the two families have been considered generally as more closely related together than to other Stylommatophora (see summary by Van Mol, 1967, and discussion by Solem, 1978). The characters invoked to establish the relationships of the two families are: 1) elasmognathous jaw; 2) digestive caecum; 3) partial separation of spermiduct and oviduct; 4) absence of ureteric internal lamellae supposed as characterizing true Sigmurethra; 5) contractility of ocular tentacles, supposedly original among Stylommatophora; 6) external peritentacular nerve less integrated into procerebrum than in other Stylommatophora.

Solem (1978) has already demonstrated the relative lack of significance of the jaw, separation of genital ducts and mode of retraction of the tentacles. No systematic importance can be given to gross features of the jaw. Its elasmognathous condition may be, in athoracophorids, related to the absence of free buccal retractor muscles. In this family the buccal mass is moved only by protractors, and the occurrence of a process prolonging the jaw may help the extrusion of the jaw without extrinsic retraction of the lower part of the buccal mass. The absence of free buccal retractors is clearly the result of limacisation, which is also the case of the reduction of the ocular retractors (Figs. 19 and 20, OR) which is related with contractility of the tentacles if Solem is right. As the external peritentacular nerve is related to tentacle retraction, its similar position in succineids and athoracophorids may be a part of the same convergence; but also the position of this nerve is known in so few stylommatophoran families that it is premature to make any definitive conclusions about its value. The separation of gonoducts occurs in several unrelated families (pupillids, helicarionids, tornatellinids, *Craterodiscus*) and is not by itself an indication of phylogenetic relationships.

The descriptions given above show that the digestive caeca found in succineids and athoracophorids are not homologous: the digestive caecum seems to be primarily an appendix of the posterior duct of the digestive gland in succineids; it seems to be primarily intestinal and secondarily an appendix of the anterior digestive duct in athoracophorids. A digestive caecum occurs at least in some Limacidae where it is an intestinal appendix (Simroth & Hoffmann, 1908–1928, pl. 19, fig. 3) and in the North American arionid *Hemphillia* (unpublished). Since these caeca are found in various positions, and since they are found only in semislugs and slugs among Stylommatophora, it is not absurd to suppose that they are secondary and not homologous of caeca found in prosobranchs and Basommatophora. In Stylommatophora these caeca may provide a compensation to the decrease of intestinal length in limacisation, or provide the equivalent of an additional length of intestine which cannot be housed in a reduced visceral space.

As shown above, the characters of the lung that justified for many authors a suborder "Tracheopulmonata" occur in some semislugs unrelated to athoracophorids. The athoracophorid lung may be interpreted as entirely formed by structures which are accessory in semislugs as parmacellids, and which completely replace the primary lung in this family.

If all possible interpretations of the an-

atomical characters of the athoracophorids proposed by Solem (1978), and here, are accepted, it is possible to establish an ideal portrait of snail and semislug ancestors of this family. Such ancestors could probably have the following characters:

1. Their shell would be flat rather than bulimoid: if bulimoid shells are related with detorsion of subsequent slugs, such hypertorted slugs as athoracophorids are more easily derived from a helicoid snail retaining hypertorsion in limacisation;
2. The pallial complex would show the usual sigmurethrous disposition in a snail athoracophorid ancestor, and would be similar to the pallial complex of *Parmacella* in a semislug or slug athoracophorid ancestor;
3. The free retractor muscles would follow the general pattern found in Stylommatophora. The penial retractor would be either a branch of the columellar retractor, or would be inserted on the wall of the foot cavity outside of the periaortic bend of the intestine;
4. The cerebroid ganglia could be of the usual stylommatophoran type, i.e. without a procerebral posterior connective joining the metacerebrum to the procerebrum. It is difficult to determine from which of Bargmann's types of suboesophageal chain the athoracophorid suboesophageal chain derives (Bargmann, 1930; Bishop, 1978), but it may be noticed that the disposition here described (Fig. 21) is very similar to the arrangement found in arionids (*Arion*, Van Mol, 1962);
5. The pedal gland would possibly lie free in the foot cavity; it would not be the case if, as supposed by Watson (1930), a free pedal gland is related to the enlargement of the foot cavity;
6. The digestive tract might have only oesophageal ridges as internal morphological differentiation (but of course the absence of crop and stomach ridges may be secondary, and more especially as athoracophorids are so modified in relation with limacisation);
7. It would be an additional indication if a snail or a slug fitting this picture occurred in Melanesia, Australia or New Zealand. It is not absolutely necessary if athoracophorid ancestors came from the north as postulated by Solem (1959).

Data are lacking to establish which aulacopod group could be considered the most closely related to such hypothetical athoracophorid ancestor, but we know enough to determine that succineids are probably less closely related to it than almost any other aulacopod family by three, possibly four characters, implying an early divergence. These characters are the bulimoid shell involving slug detorsion, the procerebral posterior connective (Van Mol, 1967), the penial retractor insertion and possibly the inclusion of the pedal gland in the foot. If one accepts the set of hypotheses here proposed, which includes the acceptance of Solem's classification (1978), one can describe the common ancestor of succineids and other Aulacopoda as having a bulimoid shell involving hypertorsion of the posterior digestive tract, a posterior procerebral connective, and penial retractor a branch of the columellar retractor. Succineids would have retained the bulimoid shell and the posterior procerebral connective, and the insertion of their penial retractor would have migrated following the line 0–01 (Fig. 14). All other Aulacopoda would have lost the procerebral posterior connective and acquired an helicoid shell but would have primarily retained hypertorsion of the posterior digestive tract. Their penial retractor would have migrated to the left, following the 0–02 way (Fig. 14). Whatever the direction of the evolution of the pedal gland arrangement, a free pedal gland is found only in this group. Athoracophorids may be interpreted as the family which is the most advanced in limacisation within the latter group.

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REFERENCES CITED

BAKER, H. B., 1941, Zonitid snails from Pacific islands. Parts 3 and 4. *Bernice P. Bishop Museum Bulletin,* 166: 205–370, pl. 43–65.

BARGMANN, H. E., 1930, The morphology of the central nervous system in the Gastropoda: Pulmonata. *Journal of the Linnean Society of London (Zoology),* 37: 1–58, pl. 1–5.

BISHOP, M. J., 1978, The value of the pulmonate nervous system in phylogenetic hypothesis. *Journal of Molluscan Studies,* 44: 116–119.

BURTON, D. W., 1962, New Zealand Land Slugs—Part 1. *Tuatara*, 9: 87–97, pl. 1–3.

BURTON, D. W., 1978, Anatomy, histology and function of the reproductive system of the

tracheopulmonate slug *Athoracophorus bitentaculatus* (Quoy and Gaimard). *Zoology Publication from Victoria University of Wellington*, 68: 1–16.

BURTON, D. W., 1980, Anatomical studies on Australian, New Zealand, and subantarctic Athoracophoridae (Gastropoda: Pulmonata). *New Zealand Journal of Zoology,* 7: 173–198.

DELHAYE, W. & BOUILLON, J., 1972, L'évolution et l'adaptation de l'appareil excréteur chez les mollusques gastéropodes pulmonés, 2, Histophysiologie comparée du rein chez les Stylommatophores. *Bulletin Biologique*, 106: 123–142.

FISCHER, P. & CROSSE, H., 1872, Etude sur les Mollusques terrestres et fluviatiles du Mexique et du Guatemala. *In* Recherches zoologiques pour servir à l'histoire de la faune de l'Amérique centrale et du Mexique, septième partie, 1, deuxième livraison: 145–300.

FOL, H., 1880, Sur le développement des Gastéropodes Pulmonés. *Archives de Zoologie expérimentale et générale,* 1ère série, 8: 103–232, pl. 9–18.

FRETTER, V., 1975, Introduction . *In* FRETTER, V. & PEAKE, J., (eds.) *Pulmonates.* Academic Press: xi–xxix.

GHOSE, K. C., 1963, Embryogenesis and larval organs of the giant land snail *Achatina fulica* Bowdich. *Proceedings of the Royal Society of Edinburgh,* section B, 68: 237–260.

GLAMANN, G., 1903, Anatomisch-systematisch Beiträge zur Kenntniss der Tracheopulmonaten. *Zoologische Jahrbücher, Abtheilung für Anatomie und Ontogenie der Thiere,* 17: 679–762, pl. 29–34.

LACAZE-DUTHIERS, H. DE, 1887, Histoire de la Testacelle. *Archives de Zoologie expérimentale et générale,* 2ème série, 5: 459–596, pl. 29–40.

MÖRCH, O. A. L., 1865, Quelques mots sur un arrangement des Mollusques Pulmonés terrestres (Géophiles, Fér.) basé sur le système naturel. *Journal de Conchyliologie*, 13: 265–283.

OBERZELLER, E., 1970, Ergebnisse der Osterreichischen Neukaledonien-Expedition 1965. Terrestrische Gastropoda, 2: Veronicellidae und Athoracophoridae. *Annalen der Naturhistorisches Museum, Wien,* 74: 325–341, pl. 1–5.

PLATE, L. H., 1898, Beiträge zur Anatomie und Systematik der Janelliden. *Zoologische Jahrbücher, Abtheilung für Anatomie und Ontogenie der Thiere,* 11: 193–280, pl. 12–17.

POIRIER, J., 1888, Etude anatomique de l'*Estria alluaudi,* nouvelle espèce de limacien africain. *Mémoires publiés par la Société philomathique à l'occasion du centenaire de sa fondation*: 135–152, pl. 13–14.

RIGBY, J. E., 1963, Alimentary and reproductive systems of *Oxychilus cellarius* (Müller) (Stylommatophora). *Proceedings of the Zoological Society of London,* 141: 311–359.

RIGBY, J. E., 1965, *Succinea putris*: a terrestrial opisthobranch mollusc. *Proceedings of the Zoological Society of London,* 144: 445–486, pl. 1.

RUNHAM, N. W., 1975, Alimentary canal. *In* FRETTER, V. & PEAKE, J. (eds.), *Pulmonates.* Academic Press: 53–104.

SIMROTH, H. & HOFFMANN, H., 1908–1928, Pulmonata. Dr. H. G. Bronns *Klassen und Ordnungen des Tier-Reichs,* 3,2,2: 1354 p., pl. 1–44.

SOLEM, A., 1959, Systematics and zoogeography of the land and freshwater Mollusca of the New Hebrides. *Fieldiana: Zoology,* 43: 1–359.

SOLEM, A., 1966, Some non-marine molluscs from Thailand, with notes on the classification of the Helicarionidae. *Spolia zoologica Musei hauniensis,* 24: 1–110, pl. 1–3.

SOLEM, A., 1978, Classification of the land Mollusca. *In* FRETTER, V. & PEAKE, J. (eds.), *Pulmonates 2A.* Academic Press: 49–97.

STEENBERG, C. M., 1925, Etudes sur l'anatomie et la systématique des Maillots. Reitzel, Copenhagen: 1–211, pl. 1–34.

TILLIER, S., 1979, *Malagarion paenelimax*, gen. nov., spec. nov., a new slug-like Helicarionid from Madagascar (Pulmonata: Helicarionidae). *Veliger,* 21: 361–368.

TILLIER, S., 1981, South American and Juan Fernandez Succineid slugs. *Journal of Molluscan Studies,* 47: 125–146.

TILLIER, S., 1982, Structures respiratoires et excrétrices secondaires des limaces (Gastropoda: Pulmonata: Stylommatophora). *Bulletin de la Société Zoologique de France,* 108: 9–19, 1 pl.

VAN GOETHEM, J., 1977, Revision systématique des Urocyclinae (Mollusca, Pulmonata, Urocyclidae). *Annales du Musée Royal de l'Afrique Centrale,* serie in-8°, sciences zoologiques, 218: 1–355, pl. 1–4.

VAN MOL, J. J., 1962, Anatomie et physiologie de la limace rouge *(Arion rufus). Les naturalistes belges*, 43: 1–17.

VAN MOL, J. J., 1967, Etude morphologique et phylogénétique du ganglion cérébroïde des Gastéropodes Pulmonés (Mollusques). *Mémoires de l'Académie Royale de Belgique*, classe des sciences, 37(5): 1–168, pl. 1–3.

VAN MOL, J. J., 1970, Révision des Urocyclidae (Mollusca, Gastropoda, Pulmonata). Première partie. *Annales du Musée Royal de l'Afrique centrale,* série in-8°, sciences zoologiques, 180: 1–234.

VAN MOL, J. J., 1971, Notes anatomiques sur les Bulimulidae (Mollusques, Gastéropodes, Pulmonés). *Annales de la Société Royale Zoologique de Belgique,* 101: 183–226.

WATSON, H., 1915, Studies on the carnivorous slugs of South Africa, including a monograph of the genus *Apera,* and a discussion on the phylogeny of the Aperidae, Testacellidae and other agnathous Pulmonata. *Annals of the Natal Museum*, 3: 107–267.

WATSON, H., 1930, *Milax gracilis* in the British Isles. Part 2, Anatomy. *Journal of Conchology* 19: 72–93, pl. 2.

WIKTOR, A. & LIKHAREV, I. M., 1980, The pallial complex of Holarctic terrestrial slugs (Pulmonata, Stylommatophora) and its importance for classification. *Zoologica Poloniae*, 27: 409–448.

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OBSERVATIONS ON THE REPRODUCTIVE SYSTEM OF THE APLYSIID *DOLABELLA AURICULARIA*

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ABSTRACT

In *Dolabella auricularia* (Lightfoot, 1786), the little hermaphrodite duct leads to a fertilization chamber where the female channel opens. The male channel continues along the large hermaphrodite duct as a ciliated groove. The fertilization chamber, placed at the base of the winding gland adjacent to the large hermaphrodite duct, also receives ducts from the albumen gland and receptaculum. It leads to the winding gland with a double helicoid spiral course. An axis of dense connective tissue supports both the inward channel, coiling clockwise, and the outward, coiling anticlockwise; the former ends in a short muscular duct which enters a vesicle at the apex of the gland and a similar duct links the vesicle to the latter. Ova, sperm and albumen coated with secretion from the inward passage pass to the vesicle where capsules are moulded singly. There is evidence that acid mucopolysaccharide, which coats each capsule and forms the chalaza joining it to the next, comes from the outward passage, moulded by a narrow duct linking winding and mucous glands. The latter coils over much of the former and also the more posteriorly placed albumen gland, making one anterior and three posterior loops; all are firmly bound by connective tissue. A tract of dense supporting tissue overlies the conducting channel of the mucous gland whose secreting area is increased elsewhere by closely-set lamellae. The lumen of the first and second loop contains a typhlosole. In parts of the gland strips of ciliated epithelium with underlying muscles join the free tips of the lamellae and these may become numerous and form an inner wall perforated for the inflow of secretion.

Within the mucous gland the capsule string is embedded in a thin coat of viscous secretion, then coiled into an irregular spiral, the coils compacted and finally surrounded by a layered outer wall. The first event occurs in the first loop of the gland where the lumen is relatively narrow. It is unknown how the coiling is accomplished, but it is completed, the coils compacted and some of the outer wall formed towards the end of the third loop. Here and in the remaining one an increased musculature compresses the layers of secretion. The oviducal channel of the large hermaphrodite duct produces the final tacky covering of the spawn. The winding gland is considered to be essentially similar in *Aplysia* and *Dolabella*, its size and robustness varying with the species.

INTRODUCTION

Dolabella auricularia (Lightfoot, 1786) was collected in two places of the Indo-Pacific, Puerto Galera (Philippines) and Suva (Fiji), where it is a sublittoral, shallow water form living where the substratum is sufficiently soft for it to burrow and leave the exhalant siphon from the mantle cavity in communication with the surface. During rough weather it burrows deeper for protection. As one walks over the substratum during the day and disturbs buried individuals they can be detected by secretion from the purple gland emitted by way of the siphon. These animals are most active at night when they feed on sea grass and algae including *Gracilaria salicornis, Padina australis* and *Sargassum* spp. When plants are not available *Dolabella* browses amongst the sandy substratum and collects smaller algal growths, foraminiferans, sponges, worms, small gastropods and ophiuroids, all of which have been found in the gut. *Dolabella* lives singly except when breeding and then it has been found in groups of ten or more. Copulation and egg laying occur throughout the year, usually when the tide is high. Copulation is frequent; the animals have been seen in pairs, not in chain formation as in *Aplysia punctata*. The spawn, which may be pale olive green, coral pink or beige, is deposited on weeds or on stones, appearing as long, complexly coiled cords attached by a sticky outer covering.

Despite the abundance of the animals and their use as food, knowledge about the reproductive system is scanty. Eales (1946) studied the gross structure of the hermaphrodite

duct in two preserved specimens of *Dolabella gigas* Rang, but their state of preservation was obviously a limiting factor in the investigation and may account for the considerable differences between her description and the one presented here. Previous to this the reproductive system and spawn of an unnamed species was figured by Hirase (1929), but he revealed no detailed structure of the gross anatomy. In contrast to *Dolabella* the reproductive system of *Aplysia* spp. is well documented, but in order to verify certain points two species were reinvestigated, *A. californica* Cooper, obtained from the Pacific Bio-Marine Supply Co., Venice, California, and *A. punctata,* obtained from the Marine Biological Association, Plymouth.

REPRODUCTIVE SYSTEM

The reproductive system is most readily exposed by making a median longitudinal incision through the foot. At its posterior limit is the compact, hemispherical, khaki, hermaphrodite gland, with connective tissue attachments to the digestive gland (Fig. 1, h). The little hermpahrodite duct is narrow as it emerges from the centre of the flat surface of the gland, but is soon distended with sperm and becomes the loosely-coiled vesicula seminalis (vs) which passes to a tightly-knit, glandular mass constituting coils of the oviduct and its associated glands. On approaching this its diameter is again reduced and an internal fold divides its lumen into male and female channels; the smaller male channel is strongly ciliated, the female is lined by a higher epithelium of ciliated and mucous cells. The duct becomes attached superficially to the glandular mass and makes a circuitous course on its way to the fertilization chamber which lies beneath the receptacular duct near its junction with the large hermaphrodite duct. It first runs parallel with this duct (d) then passes over it and loops over the exposed surface of the albumen gland (al) in reversing its direction to approach the fertilization chamber. On reaching this chamber the two channels separate: the male enters the large hermaphrodite duct and continues along it as a ciliated groove, the vas deferens, whilst the female opens to the fertilization chamber. The subsequent course of the oviduct is complex. The first part is the tightly-coiled winding gland (Fig. 2A, B, ow), of conical shape with the apex anterior, which is mainly hidden since it is covered anteriorly, ventrally and to a varying degree dorsally by the very large second part, the mucous gland (Fig. 2A, m).

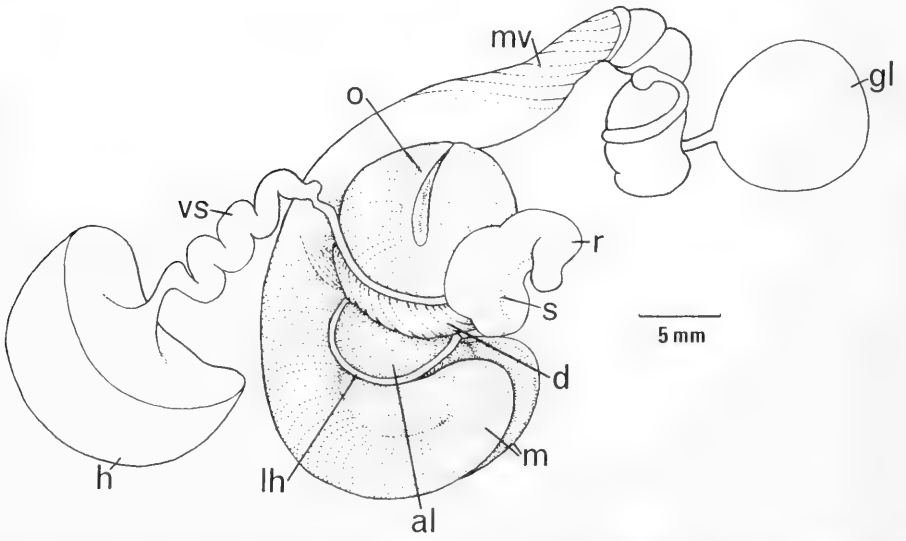

FIG. 1. *Dolabella auricularia*. Reproductive system, ventral view. Seminal groove and penis omitted. al, albumen gland; d, duct of receptaculum; gl, gametolytic gland; h, hermaphrodite gland; lh, little hermaphrodite duct; m, mucous gland; mv, muscles of vaginal channel; o, band of supporting tissue overlying ciliated conducting channel; r, receptaculum seminis; s, sperm channel leading to fertilization chamber; vs, vesicula seminalis.

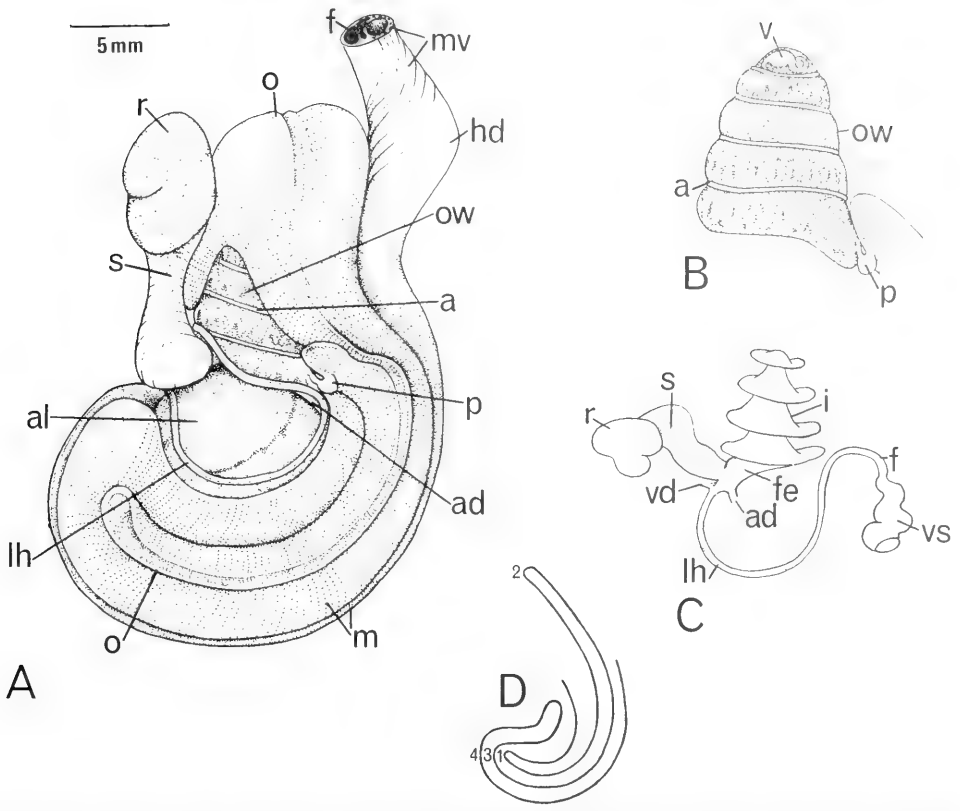

FIG. 2. A, glands and ducts of the female reproductive system in dorsal view. B, winding gland and origin of mucous gland in dorsal view. C, diagram of the connective tissue framework of the helicoid spiral of the winding gland as seen in ventral view and the fertilization chamber with associated structures. D, diagram to show the course of loops 1–4 of the mucous gland in dorsal view. a, artery; ad, duct of albumen gland; al, albumen gland; f, female channel of large hermaphrodite duct, the narrow vas deferens runs alongside; fe, fertilization chamber; hd, large hermaphrodite duct; i, inward channel of winding gland; lh, little hermaphrodite duct, the dotted line indicates division into male and female channels; m, mucous gland; mv, muscles of vaginal channel; o, band of supporting tissue overlying ciliated conducting channel; ow, outward passage of winding gland; p, passage from winding gland to mucous gland; r, receptaculum seminis; s, sperm channel leading to fertilization chamber; v, vesicle at apex of winding gland; vd, vas deferens; vs, vesicula seminalis.

The mucous gland, which leads to the large hermaphrodite duct (hd), is conspicuous not only because of its size and superficial position, but also on account of its colour—narrow longitudinal, orange bands (o) on a grey background. The areas it covers vary not only with the state of development of the reproductive system, but also with short spells of starvation or after spawning when the glandular tissue is reduced and the coils shorten.

The fertilization chamber (Fig. 2C, fe) is at the base of the winding gland adjacent to the large hermaphrodite duct and can be exposed most readily if the duct of the receptaculum is dissected away from underlying tissues. It is bordered posteriorly by the albumen gland (Fig. 2A, al) which discharges by a single duct near the opening of the female channel of the little hermaphrodite duct (Fig. 2C, ad). Sperm from the receptaculum are freed to the chamber by way of a narrow channel which runs along the length of the receptacular duct (s). The chamber is lined by ciliated epithelium and ridges along its walls lead to the entrance of the winding gland.

The albumen gland is yellow in living tissue and consists of one lobe with deeply-folded walls. It is surrounded by a connective tissue with a complex network of muscle fibres and these tissues penetrate the folds. The epithel-

ium is composed of gland cells and interspersed supporting cells that are weakly ciliated, indicating that muscles rather than cilia are responsible for directing secretion towards the strongly ciliated and muscular duct. The gland cells are uniform and filled with moderately large spherules that give the gland its colour.

The duct of the receptaculum seminis arises from the vaginal channel of the large hermaphrodite duct and crosses the base of the winding gland to which it has firm connective tissue attachments. It is prominent on account of its breadth and the thickness of its muscle bundles arranged in a loose spiral. The duct is free distally and opens to a large receptacular pouch (Fig. 1, r) that also is spirally twisted. Along its course a tract, pink in colour, marks a deep ciliated groove leading to the fertilization chamber (s).

The term winding gland refers to the helicoid spiral course of the initial part of the oviduct. The axis of the spiral is horizontal with the apex directed anteriorly. It is formed of dense connective tissue with muscle fibres that are the framework of the structure supporting both inward and outward channels, the latter overlying the former which is thus hidden from surface view. At the apex of the gland each channel narrows to a short muscular duct and the two are joined by a vesicle with muscular walls (Fig. 2B, v) which has the size and shape of an egg capsule. The ascending channel (Fig. 2C, i) leading from the fertilization chamber (fe) describes a clockwise course and the descending one (Fig. 2B, ow) leading to the mucous gland is counter-clockwise. The former is lined by a tall epithelium of gland cells alternating with ciliated cells. Its secretion is neither protein nor mucous. The ciliated cells constitute the most striking feature of the epithelium and call for detailed examination. The cilia of each cell are fused to form a sharply-pointed projection of moderate length so that the surface of the epithelium appears to be covered by minute spines suitable for the transport of highly viscous material. Fibrillae connect each compound cilium with a dense aggregation of small spherules at the base of the cell. In certain places, where the epithelium overlies the framework of connective tissue and there appears to be no basement membrane, the fibrillae penetrate this tissue for a short distance and groups from adjacent cells terminate at different levels. The overlying descending channel is more elaborate. On the outer surface there is an opaque, raised tract zigzagging along the spiral course, the base of each loop disappearing from sight as it passes inwards, making a similar course on the axial wall. Along this tract the glandular epithelium is folded, with connective tissue and muscles penetrating the folds. The gland cells here secrete acid mucopolysaccharide unlike the glands of the adjacent epithelium. All secreting cells alternate with ciliated cells resembling those of the underlying channel. The stoutest compound cilia are not those covering the folds, but those of the lower epithelium bordering them.

A short, narrow, muscular duct links the winding gland with the mucous gland (Fig. 2B, p) which is a broad duct coiling around the albumen and winding glands, the adjacent coils firmly joined to one another and to the underlying structures by connective tissue. The course of the gland is made conspicuous in fresh material by the narrow orange band (Fig. 2A, o) which marks the position of a tract of dense supporting tissue associated with a conducting channel lined by an epithelium with compound cilia. On either side of the band, in preserved material, transverse lines visible on the surface of the gland mark the attachments of closely-set dorsoventral or oblique folds which restrict the lumen. The gland describes three dorsal and one ventral loop (Figs. 1, 2A, m). The first leading posteriorly from the winding gland is encircled by the third (Fig. 2A, D), whilst the second, which is broader, is a large anterior loop overarching the winding gland. The third runs around the posterodorsal part of the albumen gland towards the base of the winding gland, then passes ventrally to join the fourth loop, which underlies the third and leads anteriorly to merge with the oviducal channel of the large hermaphrodite duct at the level of the fertilization chamber. The orange bands of the first and second loops lie close together and between them is the origin of a typhlosole which is illustrated in an oblique transverse section near the base of the anterior loop (Fig. 3). The typhlosole has a median sheet of connective tissue with muscle fibres (me), and these penetrate branching folds of the epithelium. The free edge of the connective tissue sheet joins the mid dorsal area of a thin, horizontal plate (hp) which is muscular, covered on its ventral surface by a low ciliated epithelium and is associated dorsally with the supporting tissues of the adjacent epithelial folds. This typhlosole pro-

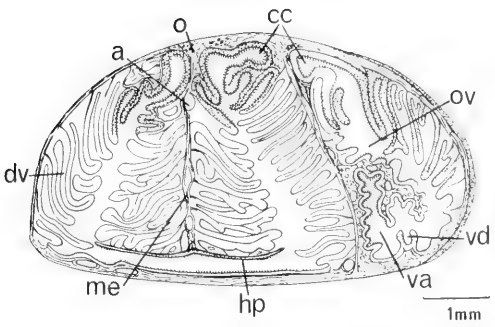

FIG. 3. Transverse section of large hermaphrodite duct not far from its origin and anterior loop of mucous gland. Stippled epithelium indicates sites of production of acid mucopolysaccharide. a, artery; cc, conducting channel; dv, dorsoventral folds; hp, horizontal plate; me, median sheet of connective tissue, o, supporting tissue with pigment granules overlying ciliated conducting channel; ov, oviduct; va, vagina; vd, vas deferens.

vides the adjacent conducting channels with greater flexibility and control of contents than a closed duct. The two channels it subdivides are enclosed by a coat of circular and longitudinal muscles from which connective tissue and muscle penetrate the lateral epithelial folds. The epithelium of the conducting channel and lamellar folds of the mucous gland consists typically of gland cells alternating with ciliated cells; over the folds the cilia are not compound. Glands secreting acid mucopolysaccharide are particularly associated with the conducting channel. At the tips of the folds ciliated cells may replace gland cells and such ciliated strips with bands of underlying muscle may join adjacent ones. Ultimately in some parts of the gland the lumen is lined mainly by a ciliated epithelium perforated for the flow of secretion from the lamellae. This arrangement is a means of supplying secretion for the spawn mass and at the same time moulding it. The moulding is a function of the muscles surrounding the mucous gland which increase along the third and fourth loops: peristaltic waves have been seen passing along these loops.

The course of the vaginal channel along the large hermaphrodite duct anterior to the duct of the gametolytic gland (Fig. 1, gl) is marked by thick bands of muscle which surround it and can be seen externally (Figs. 1, 2A, mv). The extended penis, equivalent in length to the body, passes along this open channel to the receptacular duct and its course is presumably lubricated by secretion from the glands of the vagina. Distally the penis broadens to a spatulate end, the surface of which is smooth and this enlargement relates to the breadth of the inner end of the vaginal channel. A glandular epithelium also covers the seminal groove and oviducal channel: in the former the secretion is acid mucopolysaccharide, in the latter, which has more abundant glands, it is a viscous, semiopaque fluid similar to the outermost covering of the spawn cord.

THE SPAWN

The egg cord is tangled in a compact, irregular mass each loop of the cord adhering to its neighbours and to weed or stone since the outer surface is tacky. The largest freshly-deposited mass from Puerto Galera was 90 × 60 mm and when unravelled was 30 m long. Such an egg cord may take up to 24 h to produce. It is essentially a tube 2.0–2.4 mm in diameter through which can be seen the egg capsules arranged end to end in an irregular, closely-packed spiral (Fig. 4B). The capsules are ovate when freshly laid, the average length of those from Fiji 430 μm and breadth 344 μm, and each contains typically a constant number of eggs. This number was four in spawn from Fiji and one with an occasional second from Puerto Galera. Each group or single egg is surrounded by albumen (Fig. 4A, al) which is bounded by a membranous capsule wall (m); the wall though colourless and transparent is conspicuous since it reflects light. Chalazae link one capsule to the next (c). If the wall of the egg cord is damaged unprotected capsules are not emitted from the wound for they are linearly arranged within a string of semiviscous fluid which is spirally coiled. The string can be easily unravelled by fine dissection (Fig. 4B).

A closer examination of the wall of the egg cord (Fig. 4A, w) under the outer adhesive layer (ol) shows that it is made up of thin concentric tubes, apparently indicating where successive layers of secretion have been compacted as the coiled egg string passed down the genital duct. When fresh egg cords are stained with methylene blue the innermost of these becomes purple blue, the others a light blue and a thin layer around each egg capsule, continuous with the chalazae, also stains purple blue denoting acid mucopolysaccharide.

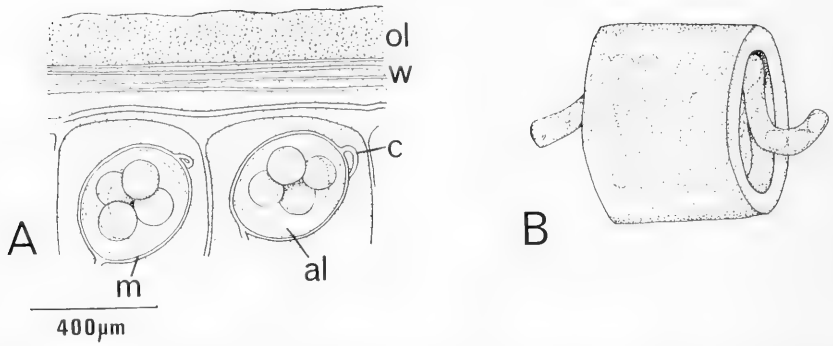

FIG. 4. A, optical section of 2 egg capsules in spawn mass. B, small piece of spawn to show spiral packing of egg capsules. al, albumen; c, chalaza; m, membranous wall of capsule; ol, outer adhesive layer; w, stratified wall.

THE FORMATION OF THE SPAWN

Only scattered observations have been made on the functioning of the complex oviduct. One dissection in Fiji revealed eggs in the winding gland: a group of four embedded in fluid in the apical vesicle and encapsuled eggs in the outward passage, a uniform number in each capsule. This indicates that the contents of the fertilization chamber are directed along the inward passage of the winding gland, coated with a secretion from its glands and a constant volume passed into the vesicle. The inward passage ends in a narrow muscular duct with the lumen occluded when the muscles are contracted, and a similar duct regulates the outlet from the vesicle. Within this chamber the capsule must be shaped and the surface layer of fluid compressed to form its wall. The abundance of acid mucopolysaccharide secretion in the outward passage suggests that this provides the mucous covering of the capsules and of the chalazae which link them into a series. The entrance to the mucous gland is by way of another narrow muscular duct through which the capsules must pass singly as their outer covering is compressed and constricted to a thread between each one.

The next stages in the formation of the spawn are the embedding of the rhythmically-produced series of capsules in a viscous secretion, the breadth of which in the final egg mass is little more than that of the capsules, then the coiling of this and the compacting of the coils. All of this occurs in the mucous gland. The first stage occurs in the first loop of the gland where the lumen is relatively narrow. Where the coiling occurs is unknown. It has been accomplished and the coils compacted by the end of the third loop and some of the outer wall has been formed. The muscles which compress the layers of secretion comprising the wall are more evident here and also along the final loop. These muscles include those surrounding the gland and beneath the ciliated epithelium at the tips of the glandular lamellae. The final tacky covering of the spawn comes from glands of the oviducal channel of the large hermaphrodite duct.

DISCUSSION

The spawn mass of the aplysiid gastropod is an elongated cord looped into a tangle as it is deposited; the outer surface is then tacky and acts as a glue in binding and anchoring it. Its colour is related to algal food and varies even between individuals of the same species (Carefoot, 1967; Chapman & Fox, 1969; Switzer-Dunlap & Hadfield, 1977). Its overall composition is uniform in that the eggs are in capsules tightly packed in spirals in the core of the cord and surrounded by an apparently stratified semitransparent wall. This uniformity indicates that the fundamental plan of the oviduct in which the spawn is manufactured must be similar throughout the group. One of the early descriptions by Eales (1921) (which has become a standard reference) was for *Aplysia punctata* and she recognised that this part of the oviduct comprised a winding gland, leading from the fertilization chamber, followed by a mucous gland, with walls thickened by glandular lamellae, which led to the

female channel of the large hermaphrodite duct. The winding gland was described as a narrow duct with glandular walls and figured as a loosely coiled tube with no special characteristics. It is figured in this form and position in the diagrammatic representation of the aplysiid reproductive system by Thompson & Bebbington (1969). Other descriptions of the gland are at variance with this. Marcus & Marcus (1957) regarded it as a series of intercommunicating pouches which connected not only with the mucous gland, but also with the albumen gland. Their figure of *Aplysia cervina* and *A. brasiliana* has recognizable features of the outward passage of the gland as seen in *Dolabella* and they state that in the species of *Aplysia* they have studied the gland cannot be unrolled as a simple tube, at least in preserved specimens. Beeman (1970) figures the gland of *Phyllaplysia taylori* as a pouch with many folds around the peripheral edge and stated that the eggs are conducted in a groove around the periphery, entering the single opening of the pouch from the fertilization chamber and leaving it to enter the mucous gland. Yet another interpretation was given by Coggeshall (1972) who described the gland in *Aplysia californica* as 5–10 blind, coiled tubes opening only into the fertilization chamber. None of these investigators has attributed the manufacture of the egg capsule to this part of the oviduct, the intricacies of which have led to these varied interpretations of what may well be a fundamentally uniform structure.

The external appearance of the helicoid winding gland of *Dolabella auricularia* with its terminal vesicle and festooned course of opaque glandular tissues regulary repeated around every coil, is so unmistakable that the presence of a corresponding structure in other aplysiids can be readily detected. Only two other species have been examined and the external appearance of the gland is similar. These are *Aplysia californica* (Fig. 5) and *A. punctata* (Fig. 6). In the former, the larger of the two species, the gland is better developed and the apical vesicle is seen when the reproductive ducts are viewed dorsally. In the latter the gland is smaller, the outgoing channel little more than one whorl, and the apical vesicle less prominent. In Fig. 6 some dissection has revealed the sinistrally coiled inward channel (i) leading to the vesicle (v) from the fertilization chamber and from this it can be seen that this channel is not intimately bound to the dextrally coiled outward channel (ow) to form a single unit as in *Dolabella*. Thus the state of our present knowledge indicates that the winding gland is essentially similar in *Aplysia* and *Dolabella*, its size and robustness varying with the species.

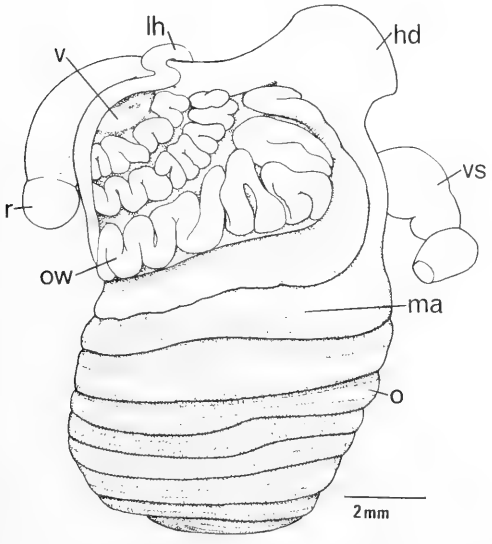

FIG. 5. *Aplysia californica,* winding gland and mucous gland in approximately dorsal view. ma, anteriorly directed limb of mucous gland passing to hermaphrodite duct. Other lettering as in Fig. 2.

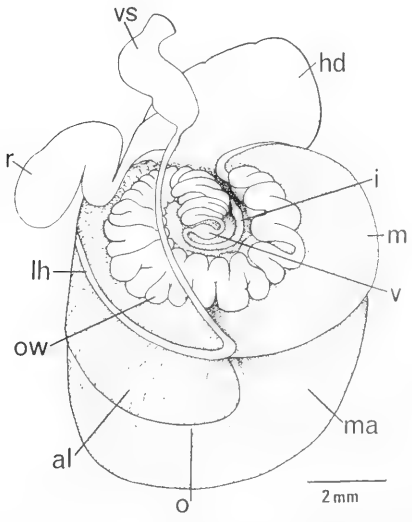

FIG. 6. *Aplysia punctata,* winding gland, mucous gland and associated structures. Coils of the outward channel of the winding gland have been separated to show the inward channel. Lettering as in Fig. 5.

The mucous gland of *Aplysia punctata* forms, from its origin, a clockwise coil, then, over the ventral surface of the albumen gland, reverses direction and envelops the base of this gland as it curves forward anticlockwise (Fig. 6, ma) to join the female channel of the large hermaphrodite duct (hd). The gland is relatively broad and short compared with that of *A. californica* (Fig. 5). In this second species it has 3.5 tightly packed clockwise coils around the albumen gland, the spiral reversing at the blind end of the gland. This pattern of coiling around the more posteriorly placed albumen gland is characteristic of the four species of *Aplysia* described by Marcus & Marcus (1957), the number of coils varying with the species. It is different in *Dolabella auricularia* which has a more voluminous gland, and reverses its direction of coiling 4 times to form 3 posterior loops around the albumen gland and one anterior overarching the winding gland; in *D. gigas* its course may be similar (Eales, 1946); indeed on examination of a good specimen may prove it to be the same. In both genera the secreting area of the mucous gland is increased by closely-set lamellar folds. In *Dolabella auricularia* these are supported by connective tissue with muscle fibres, and these tissues cover the outer wall of the gland and form a conspicuous longitudinal band visible on its exposed surface as an orange homogeneous layer. This band is the pathway for blood vessels, haemocoelic spaces and nerves and beneath it is the main conducting channel through the gland. Similar bands of supporting tissue can be traced over the exposed surface of the gland of *Aplysia punctata* and *A. californica*, and are figured in four other species by Marcus & Marcus (1957) who commented on their smooth external appearance. They are figured without comment by Thompson & Bebbington (1969) in *A. fasciata* and by Bebbington (1974) in *Phyllaplysia edmundsi*. Indeed they may be a feature of all aplysiids. Those who have studied the mucous gland of *Aplysia* make no mention of strips of ciliated epithelium along the free edges of the vertical, glandular lamellae which may link adjacent lamellae and have an underlying coat of muscles. Their occurrence in some regions of the gland in *Dolabella auricularia* is so frequent that they may function as a muscular tube within the gland.

Switzer-Dunlap & Hadfield (1977) estimated that the total number of eggs per spawn mass of *Dolabella auricularia* breeding in sea water tanks at Honolulu was 5.43×10^6. This was equivalent to the number of egg capsules. The estimated number of capsules in a spawn mass collected in Fiji was 1.4×10^6; each contained 4 eggs and the capsules were larger. The total output in these two cases is remarkably similar. The capsules are closely packed within the filamentous spawn. This is made possible by stringing the capsules together in a continuous series, the chalaza joining one to another being very short, the string is then embedded in a small amount of secretion before the packing occurs. This last event is a characteristic which has not been recorded for other aplysiids.

Dolabella auricularia lives for about a year and under laboratory conditions has a reproductive season of approximately 9 months (Switzer-Dunlap & Hadfield, 1977). During this period there may be 12 or more separate spawnings each producing about 5.5 million eggs which are 92 μm in diameter. The eggs are closely packed in a cord which may be as much as 30 m long. Such productivity is far in excess of that of any prosobranch. In that group, examples of high output associated with planktotrophic larvae are *Conus vexillum*, with an average of 1.5 million eggs per spawning, each 140 μm diameter (Kohn, 1961), and *Strombus raninus* which produces an irregularly twined egg cord not unlike that of *Dolabella*, but with only 400,000–460,000 eggs and about 20 m long (Robertson, 1959). To what extent can the high productivity of the aplysiid be related to the organization of the reproductive duct? in gonochoristic and hermaphroditic prosobranchs this duct is tied to the mantle skirt; in the opisthobranchs, concurrently with the sinking of the visceral mass and the shallowing of the mantle cavity, it has become free in the haemocoel where it has elaborated considerably giving a total volume of glandular material of the oviduct far in excess of that of any prosobranch, together with muscular and ciliary devices for forming and packing egg capsules. What is the advantage of the short life history and high productivity characteristic of opisthobranchs? Theoretically 66 million veligers could result from the spawnings of a single *Dolabella* and this dispersal phase, which may last 31 days or more (Switzer-Dunlap & Hadfield, 1977), although not apparently essential to this herbivore, must be of considerable advantage to those opisthobranchs which have a restricted carnivorous diet. Many of these carnivores

exploit a rich though relatively short-lasting supply of food which allows rapid growth and maturity and favours a short life history with high productivity.

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LITERATURE CITED

BEBBINGTON, A., 1974, Aplysiid spawn from East Africa with notes on the Indian Ocean Aplysiomorpha (Gastropoda: Opisthobranchia). *Zoological Journal of the Linnean Society*, 54: 63–99.

BEEMAN, R. D., 1970, The anatomy and functional morphology of the reproductive system in the opisthobranch mollusk *Phyllaplysia taylori* Dall, 1900. *Veliger,* 13: 1–31.

CAREFOOT, T. H., 1967, Growth and nutrition of *Aplysia punctata* feeding on a variety of marine algae. *Journal of the Marine Biological Association of the United Kingdom,* 47: 565–589.

CHAPMAN, D. J. & FOX, D. L., 1969, Bile pigment metabolism in the sea-hare *Aplysia. Journal of Experimental Marine Biology and Ecology,* 4: 71–78.

COGGESHALL, R. E., 1972, The structure of the accessory genital mass in *Aplysia californica. Tissue and Cell,* 4: 105–127.

EALES, N. B., 1921, *Aplysia. Liverpool Marine Biology Committee Memoirs,* 24: 1–84.

EALES, N. B., 1946, The anatomy of *Dolabella gigas. Proceedings of the Malacological Society of London,* 27: 109–118.

HIRASE, S., 1929, Eiablage von *Dolabella. Archiv für Molluskenkunde,* 61: 105–114.

KOHN, A. J., 1961, Studies on spawning behaviour, egg masses and larval development in the gastropod genus *Conus.* II. Observation in the Indian Ocean during the Yale Seychelles Expedition. *Bulletin of the Bingham Oceanographic Collection,* 17: 1–51.

MARCUS, E. & MARCUS, E., 1957, Notes on *Aplysia. Boletim do Instituto Oceanográfico,* 8: 3–22.

ROBERTSON, R., 1959, Observations on the spawn and veligers of conchs (*Strombus*) in the Bahamas. *Proceedings of the Malacological Society of London,* 33: 164–170.

SWITZER-DUNLAP, M. & HADFIELD, M. G., 1977, Observations on development, larval growth and metamorphosis of four species of Aplysiidae (Gastropoda: Opisthobranchia) in laboratory culture. *Journal of Experimental Marine Biology and Ecology,* 29: 245–261.

THOMPSON, T. E. & BEBBINGTON, A., 1969, Structure and function of the reproductive organs of three species of *Aplysia* (Gastropoda: Opisthobranchia). *Malacologia,* 7: 347–380.

MALACOLOGIA, 1984, 25(1): 203–219

THE CTENOLIUM OF SCALLOP SHELLS: FUNCTIONAL MORPHOLOGY AND EVOLUTION OF A KEY FAMILY-LEVEL CHARACTER IN THE PECTINACEA (MOLLUSCA: BIVALVIA)

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ABSTRACT

Study of living scallops showed that the ctenolium strengthens byssal attachment by spreading byssal threads where they pass over the disk flank. The threads converge from a cone-shaped arrangement on the substrate to a flat band over the ctenolium on the edge of the byssal notch to a cord-like mass inserted in the base of the foot inside the shell. Under tension the band of threads on the ctenolium requires a greater torque for rotation of the shell on the byssus than would a single cord. Two types of pseudoctenolium, analogous but not homologous with the true ctenolium, occur in the families Pectinidae, Propeamussiidae, and Syncyclonemidae, but the true ctenolium has been limited to the Pectinidae since the earliest known occurrence of the structure in the early Mesozoic. With congruent changes in shell microstructure, the ctenolium has helped the Pectinidae to diversify in shallow waters, in spite of increasing predation pressure since the early Mesozoic. In contrast, the Propeamussiidae are now largely limited to deeper and colder waters, where predation pressure is low, or to cryptic shallow habitats in the tropics where predation pressure is high. The Syncyclonemidae, low in diversity since the Cretaceous, barely survive at present. They have a highly disjunct distribution and are apparently cryptic and byssate. The Spondylidae, which lack a ctenolium, are moderately diverse in shallow water and apparently solved the problem of strong attachment by becoming cemented at a very early growth stage early in their phyletic history.

INTRODUCTION

The comb-like row of teeth along the ventral edge of the byssal notch of scallop shells has long attracted the attention of malacologists and paleontologists. The term "ctenolium," which is now in general use for this structure, was first applied by Dall (1895, 1898) in place of the earlier terms "pectineum" and "pectinidium," although some authors still refer to the teeth as "pectinidial."

In spite of the numerous species descriptions that refer to the presence or absence of the ctenolium and the number of teeth that it contains, there has been very little written about its development and function and even less about its taxonomic significance above the species level. The most detailed statement on development and function is still that of Dall (1898: 691):

> The right anterior [byssal notch] is usually emphasized by a flexuosity in the lower edge of the ear above it for the accommodation of the byssus, and on the upper part of the submargin are usually found a number of small, regularly spaced spines, which in life separate the threads of the byssus and thus keep it from twisting with the motion of the water. The growth of the margin of the valve and ear does not always march with the development of these spines, so that a species which normally has them may exhibit stages when the valve margin has grown over the old set and the new set has not been formed, much like the inequalities of growth shown by the margin of the aperture and the internal lirae of gastropods. . . . In old very heavy shells, which are held in place more by their own weight than by the formation of the byssus, the spines are often absent, but may usually be traced in the groove corresponding to the younger stages, or fasciole, of the [byssal notch].

It is not clear, however, whether Dall actually observed the separation of byssal threads by the ctenolium, whether such separation actually prevents twisting of the byssus, and what the consequences of a twisted byssus would be. Other functions would appear to be possible, such as protection of the byssal

TABLE 1. Key to the extant families of the Pectinacea.

A.	Ctenolium absent throughout ontogeny
B.	Prismatic calcite present on right valve ..... Propeamussiidae
BB.	Prismatic calcite absent on right valve
C.	Byssal notch absent in maturity ..... Spondylidae
CC.	Byssal notch present in maturity ..... Syncyclonemidae
AA.	Ctenolium present at least in early ontogeny ..... Pectinidae

gape when the valves are closed or as a channeling device for the movement of the foot (analogous to the columellar folds of a gastropod). Dall's description also leaves unclear the manner in which the teeth develop, whether in "sets" as he indicated, or sequentially, one tooth at a time, as with the development of spines along a radial rib on the shell exterior.

The taxonomic significance of the ctenolium has been addressed only recently. Waller (1978) subdivided the living scallops (superfamily Pectinacea) into four families—Propeamussiidae, Pectinidae, Syncyclonemidae, and Spondylidae—on the basis of a cladistic analysis of lip structure, ctenolium, shell microstructure, mantle tentacles, and cementing habit. A ctenolium was found only in species of the family Pectinidae (Table 1), and its presence was considered to be a derived character state, the primitive state being a smooth disk margin without ctenolium throughout ontogeny. More recent work by the author, however, resulted in the discovery of a ctenolium or ctenolium-like structure on all observed specimens of two rare species in the Propeamussiidae and on all known specimens of the two extant species in the Syncyclonemidae.

Do these exceptions destroy the value of the ctenolium as a key character at the family level? Specifically, if a ctenolium is indeed present in three of the four extant families of the Pectinacea, does the method of outgroup comparison dictate that the structure is primitive rather than derived? Or, do the ontogeny and detailed morphology of the structure permit the conclusion that the "ctenolium" of the Propeamussiidae and Syncyclonemidae is in fact a separate structure, functionally identical to but not homologous with a true ctenolium?

The present study attempts to answer these questions by means of a close examination of morphology, development, function, and taxonomic distribution of the ctenolium among living and fossil Pectinacea. It will be argued that although there are four distinct structures that may develop along the byssal notch, the true ctenolium is limited to and universal within the family Pectinidae. It is therefore a useful tool for tracing the phyletic history of the family.

MATERIALS AND METHODS

The disk margins of scallop shells were examined with a binocular microscope and reflected light. Selected immature Pectinidae and many thin-shelled Propeamussiidae were examined with a compound microscope and transmitted light. Specimens for scanning electron microscopy were cleaned ultrasonically in distilled water, in some cases after soaking in commercial-grade laundry bleach (5% NaOCl) for periods ranging from five to 17 hours. The specimens were then sputter-coated with carbon followed by gold palladium.

Observations on the taxonomic distribution of the ctenolium and ctenolium-like structures in the Pectinacea stem from an ongoing and as yet unpublished systematic revision of living world scallops (Pectinidae, Propeamussiidae, and Syncyclonemidae, but excluding the Spondylidae). In the course of this revision, about 400 extant, probably valid biological species have been delineated in major collections in North America and Europe. Observations on fossils are based mainly on collections of the U.S. National Museum of Natural History, the British Museum (Natural History), and the Royal Institute of Natural Sciences, Brussels, Belgium.

Live bay scallops, *Argopecten irradians irradians* (Lamarck, 1819), were observed at the National Marine Fisheries Service Laboratory in Milford, Connecticut, in April 1981. Racks of standard glass microslides were placed in culturing tanks and left overnight. Slides to which young scallops had attached were then removed and examined directly in seawater beneath a binocular microscope.

Attempts to anesthetize these specimens using 2-Phenoxethanol (Eastman Kodak Co.) and to fix them with byssus intact for later critical-point drying and scanning electron microscopy were unsuccessful. Other young specimens attached to the sides of an aquarium were observed with a hand lens. Older byssate individuals attached to other scallops or to dead shells in the culture tanks were removed with the object to which they were attached. By exerting as little tension on the byssus as possible, the byssus would remain intact and could be examined out of seawater with the binocular microscope.

Specimens illustrated by photography were coated with ammonium chloride by techniques described by Kier *et al.* (1965). Abbreviations preceding catalog numbers of illustrated specimens are as follows: IRSN, Section of Mesozoic and Tertiary Invertebrates, Royal Institute of Natural Sciences of Belgium, Brussels; MCZ, Department of Mollusks, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts; and USNM, Department of Invertebrate Zoology (Mollusks), U.S. National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Terms relevant to the description of the ctenolium are illustrated in Fig. 1. The *byssal notch* is the indentation of the right anterior auricle; the *byssal sinus* is the shallower indentation of the left anterior auricle. The track left by the byssal notch as it advances with growth is the *byssal fasciole*, which forms the ventral portion of the auricle and is separated from the *disk* by the *suture*. The *byssal gape* (or pedal gape) is the opening remaining between closed valves in the vicinity of the byssal notch. The *active teeth* of the ctenolium are those that project into the byssal gape; *inactive teeth* are those which have been isolated from the byssal gape because they have been overlapped by the leading edge of the anterior auricle during growth. Shell *height* is the dorsoventral dimension.

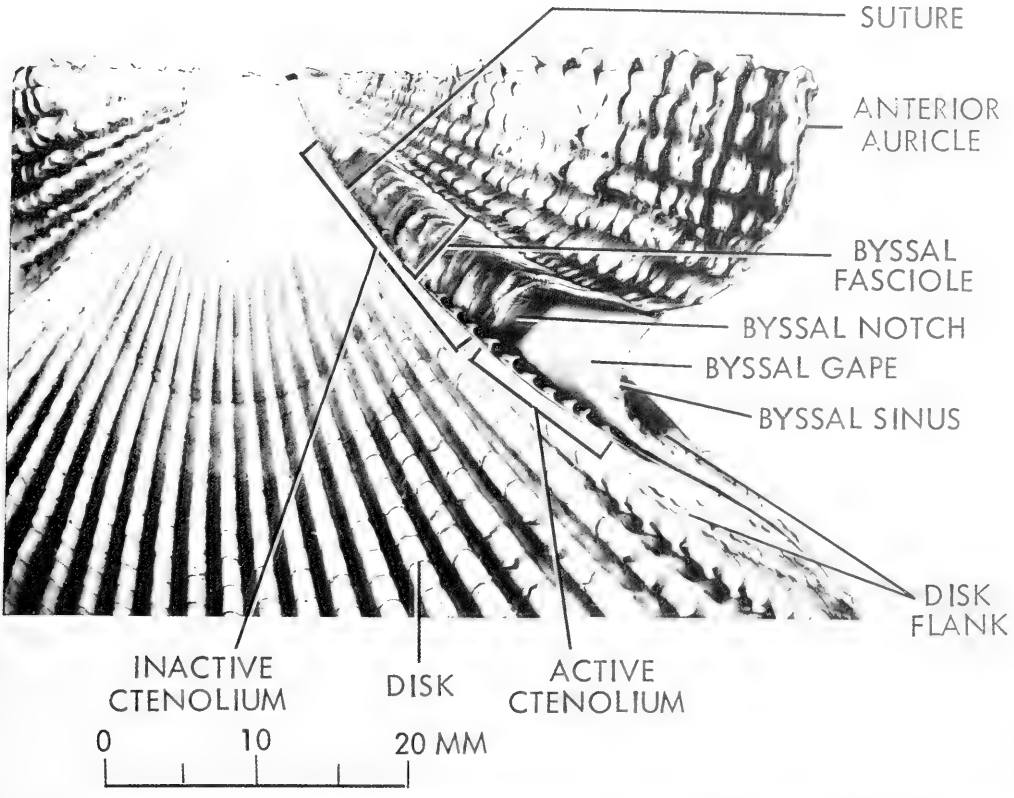

FIG. 1. Right anterodorsal region of *Chlamys senatoria nobilis* (Reeve, 1852), USNM 629232, Kii, Japan, depth 18 m, showing morphological terms used in description of ctenolium.

The shell microstructural terms *prismatic calcite* and *crossed-lamellar aragonite* were defined by Taylor *et al.* (1969). The term *lathic calcite* is used herein in preference to their "foliated calcite," because the laths comprising the structure may or may not be in side-by-side contact to produce folia, depending on the area of the shell sampled. Lathic calcite may thus be either foliated or nonfoliated.

OBSERVATIONS

Ctenolium of the right valve

Morphology and development.—In both fossil and living species of the Pectinidae, the margin of the disk along the byssal notch is generally thickened, with the inner surface of the shell turned outward and exposed in exterior view. Where most strongly developed, as in some species of *Chlamys* (Fig. 2a, b), this exposure of the inner shell surface resembles the callus or inductura of a gastropod. The teeth of the ctenolium form as elevations on the lathic calcitic growth surface of the shell on this out-turned margin. Each tooth is an elongate, arcuate, anteroventrally convex ridge. In a cross section perpendicular to the axis of each ridge, the shape is that of a hook curving anteroventrally. The ctenolium thus resembles a series of steep breaking waves passing down the thickened anterodorsal margin of the disk.

In most pectinids, the early parts of the ctenolium are preserved along the suture between the anterior auricle and disk, and a gradual increase in size and spacing of the teeth as the shell increases in size can be observed directly (Figs. 1, 2a). Also observable is the uniformity in the detailed hook-like shape of the individual teeth, the exception being the last one or two (rarely three) teeth of the active zone, which are not yet completely formed. In the vast majority of specimens studied, it is only the last tooth that lacks a hook shape (Fig. 2a).

Many pectinid shells have pigment concentrated in the outermost part of the shell, and the zone of active pigment formation is represented by a narrow band on the inner surface of the shell along the margin. Adjacent to the ctenolium, this band includes the last one or two teeth but lies on the inside of the previously formed active teeth, which are unpigmented (Fig. 2b).

The periostracum in the vicinity of the ctenolium also shows a departure from the shell margin. Shells that are well preserved have a sheet of periostracum continuous from the exterior, around the edges, onto the inner surface of the shell, ending in a thickened periostracal ridge that closely parallels the shell margin on the inner surface. This ridge is the accumulation of periostracum that was present in the periostracal groove of the mantle when the soft tissue was intact. It persists as a ridge after the soft tissues are removed. In the vicinity of the ctenolium, the ridge turns inward from the shell margin between the last one or two incompletely formed teeth and trends along the inside of the earlier teeth (Fig. 2c).

The incompletely formed ventral teeth commonly have a fresher appearance than do the more dorsal teeth. For example, a giant individual of *Chlamys townsendi* (Sowerby, 1895) from the Arabian Sea (USNM 133912, height 178 mm) has numerous perforations on the shell exterior produced by boring sponges. These borings are also present on all teeth of the ctenolium except for the last, which is not yet hook-shaped and has a fresh appearance compared to the previously formed teeth. The leading edge of the last-formed sheet of lathic calcite passes on the inside of all of the hook-shaped active teeth but then trends outward so that it encompasses the last formed tooth, which was developing on its inner surface at the time of death.

The ctenolium originates as a single tooth, not a set of teeth, very early in ontogeny but later than the byssal notch, which begins at the prodissoconch-dissoconch boundary (Fig. 2d). As shown by Waller (1972b, 1978), the outer shell layers of the early dissoconch differ in microstructure between right and left valves in most species of Pectinidae,[1] the outer layer of the right valve being prismatic calcite and that of the left being lathic calcite. With few exceptions, the prismatic calcite disappears at an early growth stage and is succeeded by lathic calcite, which previously was beneath the prismatic calcite. In some species the first tooth of the ctenolium develops at about the time that the prismatic calcite

[1]The only known exceptions are two closely related Indo-Pacific species, *Chlamys coruscans* (Hinds, 1854; see Waller, 1972a) and *Chlamys pasca* (Dall, 1908).

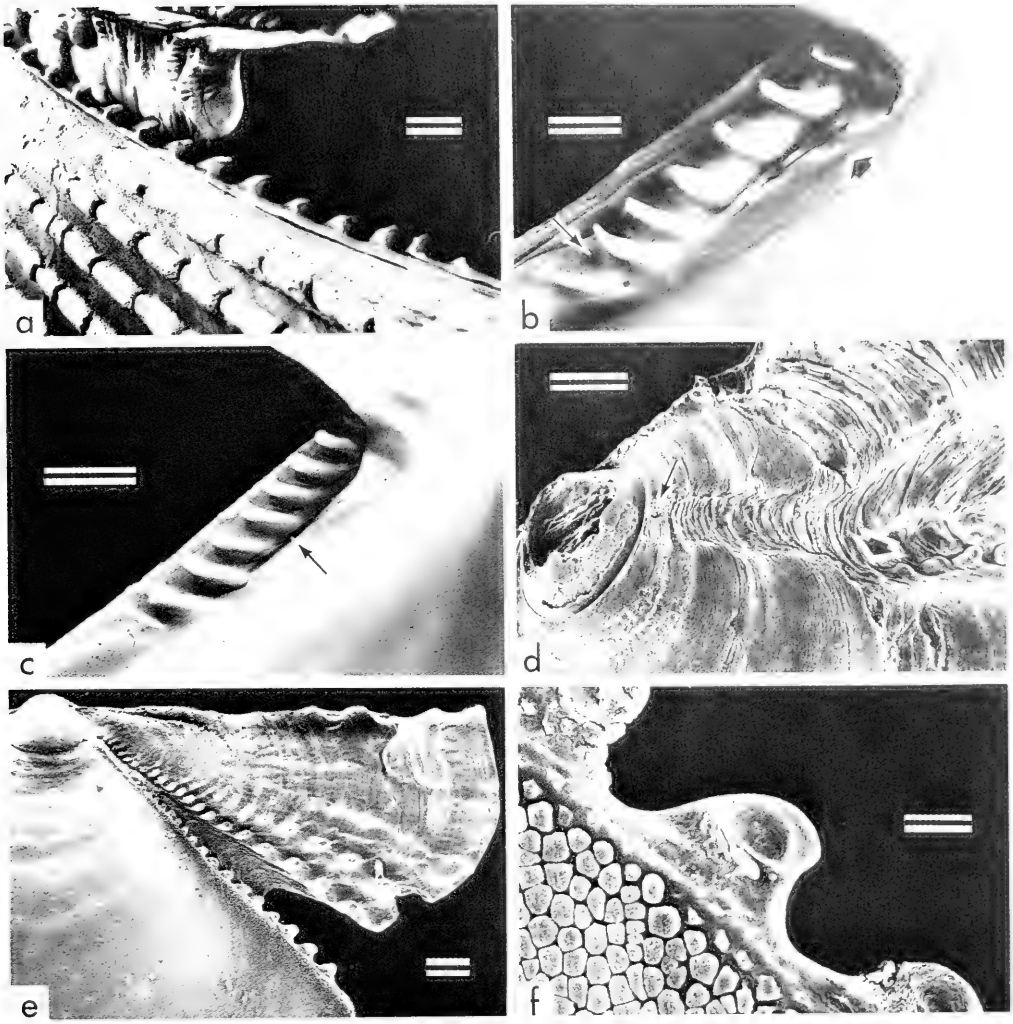

FIG. 2. Right valves of scallop shells showing byssal notch and ctenolium. a, exterior view, *Chlamys senatoria nobilis,* USNM 629232, Kii, Japan, depth 18 m, bar = 2 mm; b, interior view of same specimen showing pigmented lathic calcite inside early teeth (large arrow) and between last-formed teeth (small arrow), bar = 2 mm; c, interior view of ctenolium showing periostracal ridge (arrow), *Chlamys squamata* (Gmelin, 1791), USNM 344455, Kii, Japan, depth unknown, bar = 2 mm; d, scanning electron micrograph showing early start of byssal fasciole (small arrow) and later start of ctenolium (large arrow) on shell of young *Argopecten irradians irradians* (Lamarck, 1819), USNM 784659, culture tanks of Milford aquaculture laboratory, bar = 0.1 mm; e–f, scanning electron micrographs of anterodorsal region and detail of ctenolium of *Lissochlamis exotica* (Dillwyn, 1817), USNM 674487, Gorée, Senegal, depth 5 m, bars = 0.1 mm and 20 μm.

disappears. Many other species, however, have a well-developed ctenolium long before the microstructural change occurs, and among the few exceptional pectinids having prismatic calcite throughout growth, a ctenolium is present (Fig. 2e, f). Regardless of the microstructure of the outer layer of the disk at the time the ctenolium forms, the ctenolium itself is constructed of lathic calcite and does not involve the prismatic layer.

Among those pectinids that do not have a ctenolium throughout growth, the disappearance of the ctenolium is the result of overgrowth by the advancing front of the an-

terior auricle (Figs. 2a–c, 3a, b), as noted by Dall (1898). The process is the result of a smooth allometric relationship between the growth rate of the ctenolium and that of the right anterior auricle. Growth of the auricular margin in the byssal notch gradually increases relative to the growth of the auricular margin outside the notch; the notch gradually becomes shallower, and the length of the disk margin on which the ctenolium forms becomes shorter. Finally, the last active tooth is overlapped even before it is fully formed.

Taxonomic distribution and variation.—The number of extant, probably valid biological

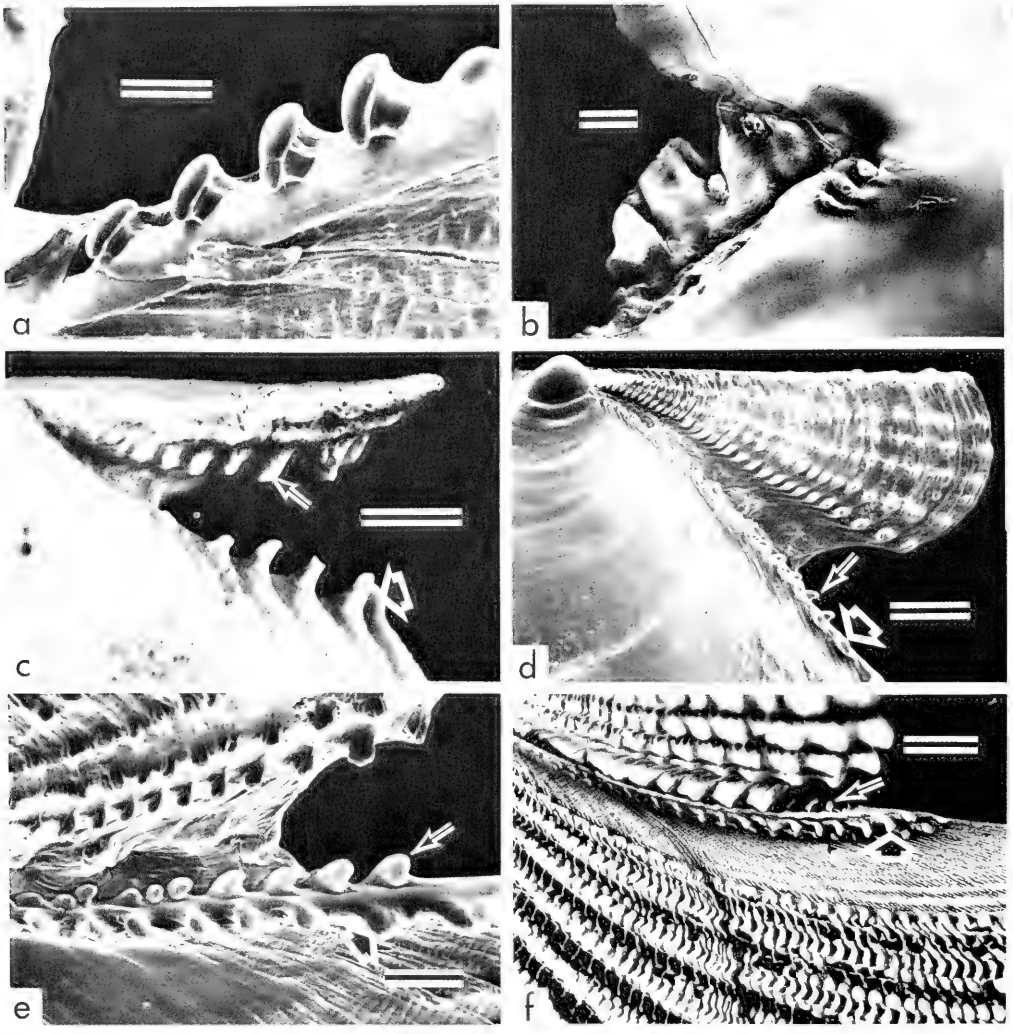

FIG. 3. a, exterior view of ctenolium of young *Argopecten irradians irradians*, shell height 4.8 mm, USNM 784660, Milford aquaculture laboratory, bar = 0.1 mm; b, interior view of byssal notch closed by exterior frills, *Chlamys (Hinnites) pusio* (Linnaeus, 1758), USNM 196525, locality unknown, bar = 1 mm; c, exterior view of disk-type pseudoctenolium (large arrow) and row of auricular spines (small arrow), *Oxytoma (Oxytoma) inaequivalvis* (Sowerby, 1819), IRSN 10203, Bathonian-Callovian age (Middle Jurassic), Ranville, France, bar = 1 mm; d–e, scanning electron micrographs of ctenolium (small arrow) and disk-type pseudoctenolium (large arrow) of *Delectopecten vitreus* (Gmelin, 1791), USNM 43708, off Nantucket Shoals, NW Atlantic, 1000 m, bars = 0.5 mm and 0.2 mm; f, ctenolium (small arrow) and disk-type pseudoctenolium (large arrow) of *Cryptopecten vesiculosus* (Dunker, 1877), USNM 36703, off Yogashima Island, Misaki, Japan, 90–150 m, bar = 1 mm.

species in the family Pectinidae thus far recognized in the course of the systematic revision in progress is 239. All of the observed individuals of all of these species have a true ctenolium at least at an early growth stage. In contrast, a true ctenolium is absent in all known extant species of the families Propeamussiidae, Syncyclonemidae, and Spondylidae.

The strength of the ctenolium, that is the number, size, and spacing of teeth relative to shell size, is highly variable among species of Pectinidae and is correlated with strength and persistence of byssal attachment. The strongest ctenolia are found among members of a diverse polyphyletic assemblage which are known to be strongly byssate, such as numerous species of *Chlamys* byssally attached to rocks or corals, *Leptopecten* attached to kelp or pilings, and *Delectopecten* attached to deep-sea sponges, corals, rocks, or the carapaces of crabs.

Ctenolia that are overgrown very early in ontogeny are found in a polyphyletic assemblage of pectinids that are known to be without byssus as adults and are efficient swimmers, notably species of *Amusium, Placopecten* (see Caddy, 1972), and the more streamlined species of *Argopecten*. Other ctenolia that disappear early in ontogeny are found among members of the genus *Pecten*, which as adults commonly live recessed in self-made depressions on muddy or sandy bottoms, swimming only when disturbed.

Between these two extremes are a number of groups that as adults are largely sedentary with weak byssal attachments but with the ability to break the attachment and swim when disturbed, e.g. most species of *Aequipecten, Argopecten,* and *Decatopecten*. Many mature individuals of species in these genera overgrow the ctenolium; maximal development of the ctenolium is in early or middle ontogeny.

Species that are unable to swim because of cementation or confinement in a crevice lose their ctenolium after becoming cemented or confined. For example, in the possibly polyphyletic genus *Hinnites*, a prominent ctenolium is present during the early "*Chlamys* stage" before cementation. Close examination of shells of *Chlamys (Hinnites) pusio* (Linnaeus, 1758) in the collections of the U.S. National Museum of Natural History and among samples received from Dan Minchin of the Irish Fisheries Division demonstrates that the disappearance of the ctenolium is not precisely correlated with the onset of cementation. Some young individuals pass through brief periods of growth during which they are temporarily cemented, and yet the ctenolium continues to form in a regular fashion. Even in mature specimens which have become permanently cemented, the ctenolium persists for a short time before being overgrown by the advancing anterior auricle. The byssal notch of some of these specimens is nearly or completely closed by exterior frills, even though the last tooth of the ctenolium is still forming and other active teeth have not yet been overgrown (Fig. 3b).

Ctenolium development in species confined to a narrow crevice is illustrated by the monotypic genus *Pedum* of the Indo-Pacific region (Yonge, 1967; Waller, 1972a). Early growth stages have a ctenolium, but confinement in later stages produces a realignment of growth, so that the byssal notch migrates through previously formed shell. The margin of the disk in the byssal notch, instead of being a zone of formation for the ctenolium, becomes a zone of dissolution.

The ecological range among species of Pectinidae is enormous in terms of both depth and temperature. Pectinids range from very shallow subtidal depths to the greatest depths known for any member of the Pectinacea (7000 m for a species of *Hyalopecten*; Knudsen, 1970) and from the warm waters of the tropics (many species) to the frigid waters of polar regions (*Chlamys islandica* (Gmelin, 1791) in the Arctic Sea and *Adamussium colbecki* (Smith, 1902) in the Antarctic). Species having a ctenolium throughout ontogeny outnumber species with a disappearing ctenolium by about five to one. Whereas ctenoliate species are distributed throughout the depth range of the family, species with a disappearing ctenolium are generally limited to depths of less than 200 m.

Function.—Living specimens of *Argopecten irradians irradians* (Lamarck, 1819) ranging in size from less than one millimeter to about 75 mm were observed at the aquaculture laboratory of the National Marine Fisheries Service in Milford, Connecticut. Young scallops in the two to five-millimeter range attached with a byssus to the upper parts of vertical partitions in the culture tanks. It appeared that the scallops reached their positions above the bottom by first swimming upward with the foot extended, the foot being large relative to size of shell at this stage. Specimens swimming into the vertical parti-

tions would adhere instantly, either by byssal attachment and/or by pedal adhesion. Most specimens would then travel further upward on the partitions by crawling, alternately extending the foot by ciliary gliding and then pulling the shell forward by contraction of the pedal retractors. Microscopic examination of glass slides on which such crawling behavior occurred showed that a succession of thin byssal threads had been secreted and abandoned during the upward travel.

Specimens still attached to glass slides had a weak byssus with only a few threads but a relatively strong ctenolium (usually with four active teeth in shells 3 mm in height or larger). There was an uneven relationship between the number of threads and number of teeth. Commonly, the several threads attached to the glass merged and passed as a single thread between any pair of teeth of the ctenolium.

Specimens of the next size class available for study, about 40 mm in diameter, were also attached with a byssus, but they were on the bottom of the culture tank (or attached to other individuals on the bottom) rather than on vertical walls or partitions. Specimens of this size were very strongly attached and could be twisted on their attachments only with difficulty, resulting in breaking of the byssus through separation of the threads from the substrate. Microscopic examination of these byssal attachments showed numerous threads attached at separate points on the substrate, the threads then merging to several compound threads and then passing over the ctenolium (Fig. 4b; see also Gruffydd *et al.*, 1979). In some cases more than one

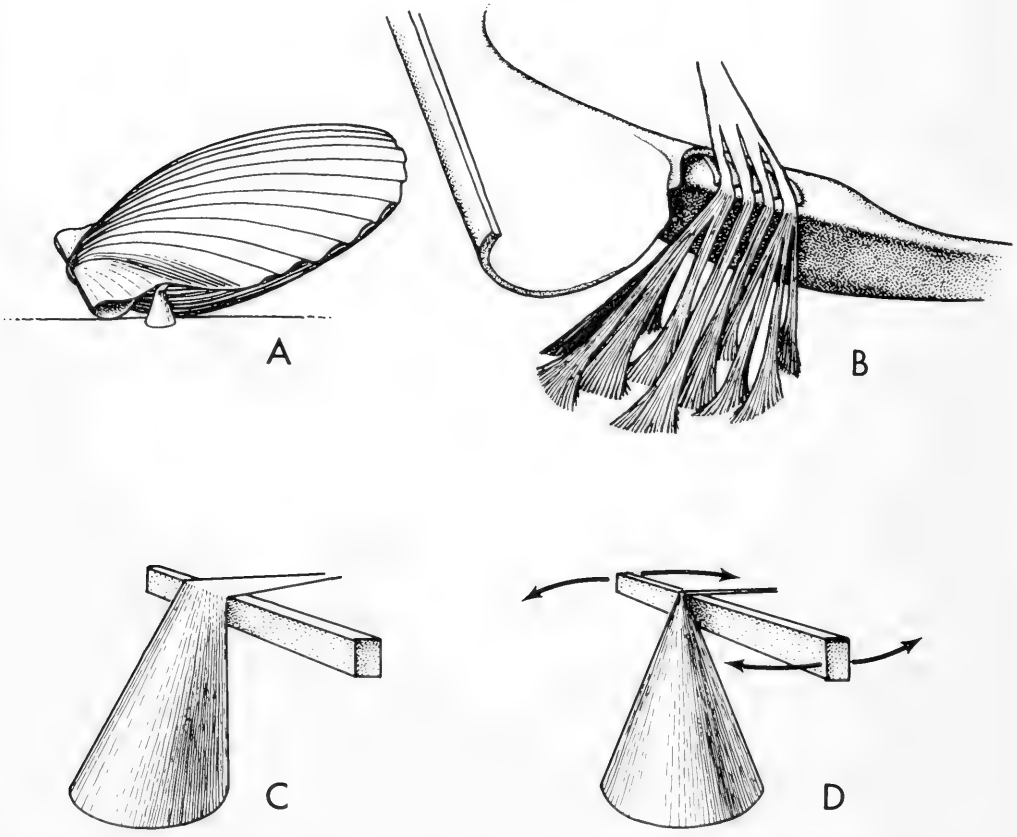

FIG. 4. Diagrams of byssus and function of ctenolium of *Argopecten irradians*. a, living position on flat horizontal surface; b, anterior auricle and left valve truncated to show array of byssal threads and relationship to ctenolium; c, cone representing array of byssal threads flattened at bar representing shell edge and ctenolium; d, same cone and bar showing that shell is more readily rotated because threads are not spread at edge of shell.

compound thread passed between each pair of teeth.

The geometrical relationship of the byssal attachment to the substrate and to the shell is shown diagrammatically in Fig. 4a–d. When the foot is retracted, the byssus is drawn tight, pulling the shell against the substrate at two points of contact, one at the tip of the right anterior auricle, the other on the anterior half of the disk (see Stanley, 1970: 31, and Waller, 1972a: 238). The byssal threads converge in a conical array from a circular or elliptical array of attachments to the substrate and merge into a flattened band as they pass over the ctenolium, whence they bend at nearly a right angle and pass as a single massive strand into the interior of the shell to their insertion point in the heel of the retracted foot.

Older, mature individuals in the size range 50 to 75 mm were observed lying free on the bottom of the culture tanks, only two of 15 specimens being attached to the bottom by a weak byssus. These attached specimens were at the lower end of the size range (diameters of 54 and 55 mm). The ctenolium of the smaller of the two had already been overgrown by the anterior auricle; a ctenolium with three active teeth was still present on the larger specimen.

Ctenolium of the left valve

A ctenolium is known to develop on the left valve only in the pectinid genus *Juxtamusium* Iredale, 1939, represented by two extant Indo-Pacific species, *J. maldivense* (Smith, 1903) and *J. coudeini* (Bavay, 1902.)[2] The ctenolium of the left valve is morphologically like that of the right valve, the teeth being hook-shaped and formed on the outwardly curved, thickened inner surface of the shell along the byssal sinus (figs. 114 and 117 in Waller, 1972a). In both species a more prominent ctenolium is present on the right valve than on the left. The ctenolium of each valve is overgrown by the corresponding auricle before maturity, but overgrowth of the left ctenolium precedes that of the right. There is also a difference in the time of origin of the ctenolium between the two species (Waller, 1972a), the left ctenolium of *J. coudeini* appearing later in ontogeny and disappearing earlier than that of *J. maldivense*.

The pseudoctenolium

The pseudoctenolium is a row of spines or teeth close to the suture between disk and anterior auricle but not formed on the "inductura" or outward-turned inner surface of the disk. There are two sites at which such a structure may form, one on the external surface of the disk adjacent to the suture and byssal notch, the other on the surface of the right anterior auricle on the leading edge of the byssal fasciole, near the suture. Rows of spines may also develop on the anterior auricle dorsal to the byssal fasciole (Fig. 3c–e), but these seem to have no functional relationshp to the byssus and are not considered further.

The pseudoctenolium of the first type, formed on the disk, is an element of external sculpture consisting of a radial row of spines very close to the suture (Fig. 3c–f). Each spine forms as an outgrowth of the shell margin like other spines on shell exteriors. The prominence of this type of pseudoctenolium may or may not correlate with the strength of other external sculpture. In several species of *Delectopecten,* for example, the exterior of the right valve is nearly smooth, and yet a pseudoctenolium originates very early in ontogeny (in some cases earlier than the true ctenolium) and persists throughout life (Fig. 3d, e). In contrast, among the two discontinuous genetic variations of the shell of *Cryptopecten vesiculosus* (Dunker, 1877) described by Hayami (1973), a pseudoctenolium is found only in the coarsely sculptured variant (Fig. 3f).

Among the extant families of the Pectinacea, the disk-type pseudoctenolium is present only in the family Pectinidae. It is also present, however, in an extinct superfamily, the Buchiacea of the Jurassic (Waller, 1978), e.g. in the genera *Oxytoma* (Fig. 3c) and *Aucellina*.

The function of a pseudoctenolium on the disk is unknown, because as yet no one has observed detailed living habits of species having the structure. There does not seem to be any correlation between the occurrence of this type of pseudoctenolium and the strength of byssal attachment. Although the feature occurs only among byssate species, it is more prominent among presumably weakly bys-

[2] *Juxtamusium coudeini* (Bavay, 1902) was referred to by Waller (1972a) as *J. oblectatum* Iredale, 1939. Examination of the holotype of *J. coudeini* in the Royal Institute of Natural Sciences of Belgium, Brussels, showed that the two species are synonymous and that Bavay's name has priority.

sate members of the *Aequipecten* group, e.g. *Cryptopecten, Corymbichlamys,* and *Aequipecten,* than among strongly byssate members of the *Chlamys* group.

The second type of pseudoctenolium forms on the auricular side of the suture and is a much more subtle feature, because it is not marked by erect spines. Rather, the structure consists of tiny, low, distally pointing projections on the edge of the auricle, nearly in contact with the suture (Fig. 5a–e). Because these projections are nearly in the plane of the auricle, they tend to be obscured as the auricle grows around them. The form and spatial relationships of the suture are nonetheless close to those of a true ctenolium. If

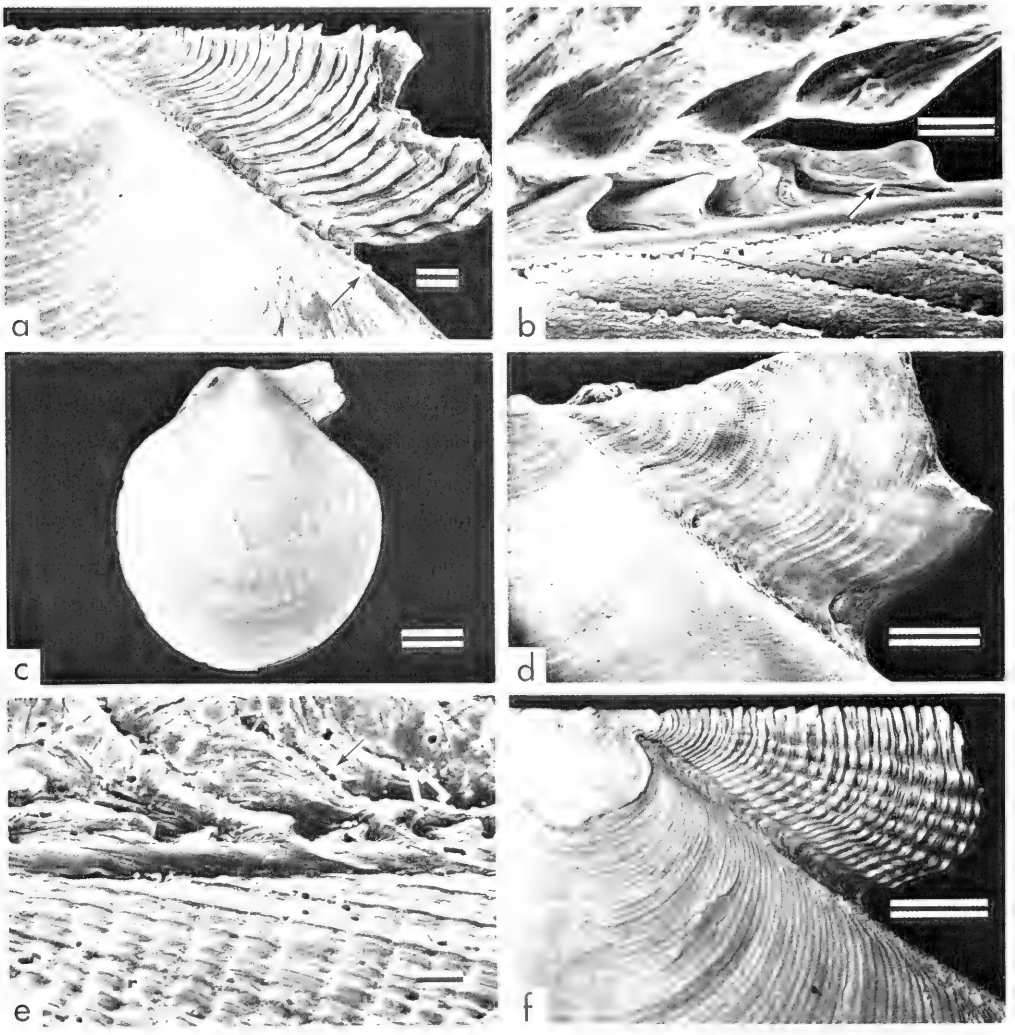

FIG. 5. a–b, scanning electron micrographs of anterodorsal region of right valve and detail of fasciolar pseudoctenolium (arrow) of *Parvamussium sayanum* (Dall, 1886), USNM 503311, Barbados, depth unknown, bars = 0.2 mm and 50 μm; c, holotype of *Syncyclonema sigsbeei* (Dall, 1886) consisting of matching valves deposited in separate collections, MCZ 7817 (right valve) and USNM 62263 (left valve), off Havana, Cuba, 290 m, bar = 2 mm; d–e, scanning electron micrographs of anterodorsal region of right valve showing teeth of fasciolar pseudoctenolium (large arrow in e) formed along auricular growth lines (small arrow in e), *Syncyclonema sigsbeei,* USNM 457911, Bay of Pigs, Cuba, 200–275 m, bars = 0.5 mm and 20 μm; f, anterodorsal region of right valve of *Cyclopecten alaskensis* (Dall, 1872), USNM 224260, southeast of Akutan Island, Alaska, 132 m, bar = 1 mm.

the "inductura" of the disk margin is continuous onto the leading edge of the auricle, then there would be only a small separation between the two types of structures. The fasciolar pseudoctenolium, however, has never been found in conjunction with a true ctenolium and appears to differ from a true ctenolium in never having more than one active tooth.

The best example of a fasciolar pseudoctenolium is found on the shells of *Parvamussium sayanum* (Dall, 1886), a poorly known species that has seldom been reported since its original description. Among the eight right valves (heights from 6.9 to 11.8 mm) in the U.S. National Museum of Natural History and the Museum of Comparative Zoology, none has a pseudoctenolium with more than one active tooth (Fig. 5a, b). The teeth are generally sharply rounded, strongly inclined toward the opening of the byssal notch, and when active are very close to the apex of the notch. Scanning electron microscopy showed that the teeth are indeed associated with the auricle rather than with the "inductura" during their formation, as indicated by the trace of growth lines from the surface of the auricle to the teeth (Fig. 5e). In some cases the teeth are unevenly spaced and one tooth is in contact with the preceding one, meaning that even if both teeth had been active at the same time, byssal threads would not have been able to pass between them. There is also an indication that some of the teeth have been superimposed on a previously formed inductura during the advance of the auricle (Fig. 5b). The inductura itself is an exceedingly narrow zone, because the prominent prismatic calcitic outer layer of the shell, characteristic of all members of the Propeamussiidae, is present to the edge of the shell, and there is very little outward turning of the lathic calcitic inner layer.

In the family Syncyclonemidae, both extant species, *Syncyclonema sigsbeei* (Dall, 1886) of the Caribbean and Gulf of Mexico and an undescribed species of *Syncyclonema* from the Pacific, have a weak fasciolar pseudoctenolium (Fig. 5c, d) and lack a true ctenolium, even though both species have a well-developed byssal notch throughout ontogeny. A notable feature of these species is the narrowness of the growth surface of the lathic calcitic outer layer of the shell. On a valve 16 mm in height, the width of the lathic calcite band on the inner surface of the shell next to the midventral margin is only 112 μm, and it is even narrower along the byssal notch. It is possible, therefore, that there is insufficient lathic calcite for the development of a true ctenolium.

The fasciolar pseudoctenolium, so far as known, is limited to the families Propeamussiidae and Syncyclonemidae and has been found in only one species besides those mentioned above. It is the propeamussiid *Cyclopecten culebrensis* (Smith, 1885), a western Atlantic species thus far known only from its type lot. The available ecological data for these species do not suggest any unusual living habits compared to those of other scallops. *Parvamussium sayanum* has been dredged from depths ranging from 165 to 732 m, with one dead valve coming from 1256 m. Dead valves of *Cyclopecten culebrensis* were dredged from a bottom of pteropod ooze at 713 m. Syncyclonemids thus far have been dredged at depths ranging from 122–411 m. Specimens from Jamaica were collected by submersible from the deep reef front at 122 m, and specimens from Hawaii are from areas where submarine ledges are know to be present (T. A. Burch, personal communication). All of these species are probably byssate, as evidenced by their byssal notch and byssal gape.

Byssal notch without ctenolium or pseudoctenolium

Among the living Propeamussiidae, 164 of the 166 species thus far recognized as valid are without either a ctenolium or pseudoctenolium. All species in the family have a prismatic calcitic outer layer on the right valve which is persistent throughout ontogeny and which commonly approaches the edge of the disk in the byssal notch. The disk margin in the byssal notch, however, is lathic calcite, just as in the Pectinidae.[3]

The ecological range of the Propeamussiidae is nearly as vast as that of the Pectinidae. Most species live at depths greater than 150 m, but there are also many in

[3]In some propeamussiids, e.g. in some individuals of *Similipecten similis* (Laskey, 1811) of the eastern Atlantic, the prismatic layer extends as a thin, transparent flange into the byssal notch, even though the byssal notch remains deep. The edge of the byssal fasciole advances beneath this flange, the flange then becoming an appliqué on the surface of the fasciole. The prismatic flange is very flexible and presumably would not interfere with the passage of the foot and byssal threads through the notch.

shallow water. Contrary to the widely held idea that most propeamussiids are free living, most species have a persistent byssal notch throughout ontogeny (Fig. 5a, f) and are therefore probably byssate. Among the deepest propeamussiids, members of the genus *Catillopecten* Iredale, 1939, are confined to depths greater than 1000 m in the Atlantic, western Pacific, and Indian Oceans and greater than 2600 m in the eastern Pacific; all have a deep byssal notch throughout ontogeny. Among the species in shallower water, those in the low and middle latitudes tend to be of very small size. For example, I observed *Cyclopecten nanus* Verrill & Bush, 1897, attached with a byssus to grains of sand nearly as large as the shell. I also found an undescribed species of *Cyclopecten* related to *C. thalassinus* (Dall, 1886) among accumulations of *Halimeda* grains on the deep reef front at Belize at depths greater than 30 m. It appears that this species attaches with a byssus to the *Halimeda* grains, and indeed its small, opaque white, flattened, somewhat polygonal shell resembles the grains on which it lives. The relatively few species (ten in the World Ocean) that have a disappearing byssal notch all belong to the genus *Propeamussium sensu stricto* and are found on muddy bottoms over a wide depth range (150 to over 2000 m).

Modern members of the Spondylidae are cemented by their right valve to a substrate throughout most of ontogeny. Members of the genus *Spondylus* at an early growth stage have a deep byssal notch but no ctenolium. In contrast, the monotypic genus *Corallospondylus* Monterosato, 1917 of the Mediterranean becomes cemented immediately after metamorphosis, and no byssal notch ever develops (Waller, 1978). The shell structure of spondylids is similar to that of syncyclonemids. Lathic calcite is present on the exterior and along the byssal noch but is confined to a narrow band on the shell interior because of encroachment by an extensive crossed-lamellar aragonitic inner layer.

Fossil record

Scallops have a rich fossil record, because their partly calcitic rather than completely aragonitic shells resist solution. Among Cenozoic scallops, the family-level distribution of the ctenolium is the same as at present. Members of the Propeamussiidae, Syncyclonemidae and Spondylidae lack a ctenolium at all growth stages, whereas all members of the Pectinidae thus far examined have a ctenolium. The distribution of pseudoctenolia has not been surveyed, but it is known that the Pleistocene representatives of the extant Pacific species of *Syncyclonema* have a pseudoctenolium like that of the modern forms.

In the Mesozoic, members of the Propeamussiidae, ranging in age from Triassic through Cretaceous, are like their later counterparts in lacking a ctenolium; no pseudoctenolia have yet been observed. The common upper Cretaceous species *Syncyclonema simplicia* (Conrad, 1860) lacks a ctenolium but has a pseudoctenolium similar to that of its later counterparts. All Mesozoic spondylids thus far examined also lack a ctenolium, and so also do members of the extinct family Neitheidae.

Most Cretaceous species that can be classified with the Pectinidae on the basis of their abbreviated prismatic calcite layer on the right valve differ from later pectinids in having an extensive crossed-lamellar aragonitic inner layer that extends well outside the pallial line and approaches the shell margins as in the other three extant pectinacean families. Such species, which have been placed in the genera *Camptonectes, Radiopecten,* and *Chlamys* (see Hertlein, 1969), all have a well-developed ctenolium. Some other Cretaceous scallops resemble modern Pectinidae more closely in that they not only have a ctenolium, but also have a reduced crossed-lamellar layer restricted to the region inside of the pallial line, e.g. species placed by Dhondt (1973) in the genus *"Lyropecten."* The placement of *Entolium*, a genus ranging from the Triassic to the Cretaceous (Hertlein, 1969), is enigmatic. Cretaceous members of the genus, but possibly not the Jurassic members, seem to have reduced crossed-lamellar aragonite. The byssal notch disappears so early in ontogeny that it has not been possible to find early growth stages finely preserved enough to allow determining whether a ctenolium is present.

There are as yet few data on the distribution of the ctenolium among older Mesozoic Pectinacea. The Triassic genus *Pleuronectites*, placed by Newell (1969) in the Aviculopectinidae (now a superfamily Aviculopectinacea, Waller, 1978), has a well-developed ctenolium that appears to be like the ctenolium of the Pectinacea. If it can be

shown that the resilium of *Pleuronectites* is of the pectinacean type (Waller, 1978), the genus would be the earliest unequivocal member of the Pectinacea. If, on the other hand, the resilium is indeed of the fibrous type found in the Aviculopectinacea, then an interesting incongruency arises in an analysis that indicated that the Aviculopectinacea are a sister group of the Pectinacea (Waller, 1978).

No Paleozoic aviculopectinaceans are known to have a ctenolium. Neither does the genus *Pernopecten*, a probable precursor of the Propeamussiidae (Waller, 1972b, 1978).

DISCUSSION

The sequential development of teeth in the ctenolium seems to be the result of mantle withdrawal from all active teeth except those still forming. This is indicated by the trends of the outer pigmented band, the periostracal ridge, and the last-formed sheet of lathic calcite, as well as by evidence from gerontic individuals that all teeth except the last have been exposed to the external environment. The mantle edge is very mobile along the margins of most bivalves (Clark, 1974; Waller, 1980), and in most cases the periostracum remains inserted in the periostracal groove of the mantle as the mantle edge withdraws from the margin of the shell. It is clear from inspection of the ctenolium by both light and scanning electron microscopy that the same happens in the region of the ctenolium, the excess periostracum being pulled over the teeth as the mantle withdraws. What is unusual here is that the withdrawal appears to be permanent adjacent to the fully formed active teeth. Functionally, this would appear to serve two purposes. First, it permits a particular tooth shape to be genetically programmed; once the shape is complete, no further calcification during growth of the shell alters it. Secondly, the withdrawal of the mantle leaves a calcified, periostracum-covered surface over which byssal threads may move without injury to the fragile tissue of the underlying mantle.

At least three functions may be hypothesized for the ctenolium of the Pectinidae: (1) it separates byssal threads and deters twisting and severing of the byssus; (2) it protects the byssal gape when the valves are closed; and (3) like the columellar folds of a gastropod, it forms a trackway on the medial side of the aperture for the passage of the foot.

It is unlikely that the ctenolium protects the byssal gape, because the teeth project over only a small part of the gape even when the valves are closed. Furthermore, even if the teeth were of a shape and size sufficient to deter small predators from entering the byssal gape, they would then also deter the worm-like foot from passing through the same opening and would also interfere with the complex and extensive movement of the foot after it is extended.

The thickened margin of the disk on which the ctenolium forms was compared earlier to the inductura of a gastropod, and it would be tempting to draw an analogy between the ctenolium and the columellar folds of a snail. Both structures are on the inside of an aperture and parallel to the movement of the foot as it slides in and out of the interior of the shell. In the scallops observed, however, complex sinuous foot movement generally occurs while the valves are gaping, and there is sometimes little contact between foot and ctenolium. Furthermore, the distinctive hook shape of the teeth seems unrelated to foot movement.

Observations of living bay scallops establish that the ctenolium indeed separates byssal threads where they pass over the disk margin, as indicated by Dall (1895, 1898). The threads can slip over the teeth in a ventral direction, away from the apex of the notch, but once tension is applied during pedal retraction, they cannot slip in the opposite direction because of the hook-like shape of the teeth. The threads are thus kept from bunching together in the apex of the byssal notch.

As observed above, there is no one-to-one correlation between the number of threads and the number of teeth. More than one thread may pass between a pair of teeth, while some spaces between teeth may have no threads. Further, some of the threads may be twisted between their attachment to the substrate and their insertion in the foot. The real advantage to spreading the threads where they pass over the disk margin under tension is to increase the amount of torque necessary to break the byssal attachment (Fig. 4c, d). The ctenolium thus allows the scallop to maintain its position relative to food-bringing currents. Probably more importantly, it allows the scallop to resist predators, such as fish or crabs, that would twist the shell on its byssus and break the attachment.

The occurrence of a ctenolium on the left valve is a rare phenomenon, found in only two closely related species in a total of about 240 species of living ctenoliate pectinids and many more in the fossil record. Although the living habits of *Juxtamusium* have not yet been observed the identical form of its left ctenolium to that of the right valve suggests a similar function. This implies that at least during that part of ontogeny in which both ctenolia are present, *Juxtamusium* may live in a crevice or other situation in which it could attach byssal threads to surfaces both to the right and left of its shell (Waller, 1972a).

The disk-type pseudoctenolium forms in a substantially different way than does the true ctenolium in that its formation involves a part of the mantle not in the byssal notch and not necessarily in contact with the byssus. This structure is an external sculptural feature, and whether it has any function cannot be answered at present. Like sculptural features, the evolution of the disk-type pseudoctenolium is iterative, as evidenced by the fact that it is found at different times in two superfamilies, the Pectinacea and the Buchiacea.

In contrast, the mode of formation of the fasciolar pseudoctenolium is very similar to that of a true ctenolium, the difference being that the former never appears to have more than one active tooth at a time and is developmentally coupled with the auricle rather than with the margin of the disk. Although the teeth of this type of pseudoctenolium form sequentially, just as the teeth of the true ctenolium, they are not associated with mantle withdrawal. Functionally, the fasciolar pseudoctenolium clearly is able to hook byssal threads. Because of the proximity of the single active tooth to the apex of the byssal notch, however, it is unlikely that any additional strength of attachment would be achieved. Instead it may be that the active tooth, which is sharper and more inclined than are the teeth of a true ctenolium, may serve to keep the entire byssus from jamming against the apex of the notch and interfering with the growth of the auricular margin. The limited taxonomic distribution of the fasciolar pseudoctenolium among the two families of the Pectinacea that have primitive characters suggests that the structure may be a largely unsuccessful evolutionary experiment with a ctenolium-like structure. There is no developmental or paleontological evidence to suggest that a true ctenolium evolved from a fasciolar pseudoctenolium.

Observations presented here sustain the previous conclusion (Waller, 1978) that a ctenolium is limited to one pectinacean family, the Pectinidae. Outgroup comparison indicates that the non-ctenoliate condition is the more common state of the byssal notch and supports the conclusion that the ctenolium is a derived character state. But if the structure is of such importance in strengthening byssal attachment, and if the overlap in living habits between the Propeamussiidae and Pectinidae is as extensive as outlined above, why is it that the ctenolium evolved only in the Pectinidae? There are possibly two reasons, one involving the complexity of the structure, the other involving linkage with shell microstructure.

The argument that a ctenolium evolved only once because of its complexity is not an inherently strong one, because the repetitive hypersecretion by the mantle that produces a ctenolium does not appear to be substantially different from that which occurs elsewhere on the mantle edge to produce exterior sculpture, which is known to be highly iterative in evolution. There are merits, however, to the microstructural argument. It has previously been shown that the relatively thick prismatic calcitic outer layer of the right valve in the Propeamussiidae has a sculpture-dampening effect, a similar effect occurring in pectinids having a prismatic phase in early ontogeny (Waller, 1972b). It may be that the mantle-edge specializations required for ctenolium formation in the Pectinidae are incompatible with the specializations required for secretion of prismatic calcite.

In the case of the Syncyclonemidae, which lack prismatic calcite and also lack a true ctenolium, there may be another sort of coupling with shell microstructure. The width of the zone of secretion of lathic calcite in *Syncyclonema* is exceedingly narrow, and the lathic calcite is succeeded on the shell interior by an extensive crossed-lamellar aragonitic inner layer (Waller, 1978). Because the ctenolium is known to form only from lathic calcite, it may be that in the Syncyclonemidae thickening of this layer would have to evolve before a ctenolium could form. In the Spondylidae, the lathic calcite outer layer is sufficiently extensive to permit development of a ctenolium. Members of this family, however, appear to have evolved a cementing habit early in their history, before the evolution of a ctenolium

In the order Pterioida, which is only dis-

tantly related to the order that contains the scallops, the Ostreoida (Waller, 1978), alate monomyarians such as *Pteria* and *Pinctada* also have a byssal notch lacking a ctenolium. In these groups thick prismatic calcite dominates the exterior of both valves, but in addition the nature of the byssus appears to be different. The byssus either resists rotation by being very massive and strong, as in *Pinctada*, or by attachment to a flexible substrate, as is the case with *Pteria* attached to flexible alcyonarian colonies (Stanley, 1970).

The evolution of shell microstructure among the families of the Pectinacea has been viewed as a response to increasing predation pressure since the Paleozoic (Waller, 1972b). The Pectinidae, having become independent of the sculpture-dampening effect of prismatic calcite and having elaborated the lathic calcitic outer layer, gained a selective advantage at all depths but particularly in shallow waters, where they were able to diversify in spite of increasing predation intensity. The Propeamussiidae, with their more fragile shell microstructure and sculptural limitations, were at an adaptive disadvantage in shallow waters and were able to diversify only in deeper water. As mentioned in the present study, propeamussiids indeed live in shallow water. In tropical regions, however, where predation is most intense (Vermeij, 1978), extant shallow-water propeamussiids are generally very small and occupy cryptic or specialized habitats, such as the interstitial habit in *Halimeda* grains mentioned above. The Spondylidae and Syncyclonemidae, without prismatic calcite but with thin lathic calcitic layers and relatively thick crossed-lamellar aragonite that extends nearly to the margins, have gained refuge in shallow water through cementation and cryptic living habits, respectively.

If a prominent ctenolium at some stage of ontogeny is indeed a derived character state unique to the family Pectinidae, as I believe it to be, then an unanswered question in pectinacean taxonomy can be resolved. It was pointed out by Waller (1978) that a great many Mesozoic scallops resemble extant Pectinidae except that they have prominent crossed-lamellar aragonite well outside of the pallial line as in modern Syncyclonemidae, Spondylidae, and most Propeamussiidae. The family-level placement of the Mesozoic taxa was left open. All, however, have a strong ctenolium, and it now appears that they are early members of the family Pectinidae. This means that crossed-lamellar aragonite retreated in many taxa to the area within the pallial line in the Cretaceous and early Cenozoic. The lack of such retreat, as well as the absence of a functional ctenolium, are thus shared primitive characters in the Propeamussiidae, Spondylidae, and Syncyclonemidae and by themselves do not signify close relationship between these groups.

SUMMARY AND CONCLUSIONS

The ctenolium consists of teeth that form sequentially as upgrowths from the growth surface of the lathic calcitic layer, which in the vicinity of the byssal notch is turned outward somewhat like the inductura of a gastropod. The hook-shaped form of each fully formed tooth is not altered by subsequent shell secretion, because the mantle withdraws from the teeth once they are fully formed, pulling periostracum over them. The ctenolium begins in ontogeny as a single tooth after the formation of the byssal notch in the early dissoconch and either persists throughout growth or is overlapped by the advancing anterior auricle and disappears. In the latter case, the byssal notch gradually becomes shallower, finally disappearing entirely in some taxa such as *Amusium* and some *Pecten*.

The function of the ctenolium is to separate the threads of the byssus by hooking the threads and preventing their accumulation in the apex of the byssal notch. The flat band of byssal threads passing over the disk flank when the foot is retracted is more resistant to rotational forces on the shell than would be a cord-like narrow strand of threads. The byssal attachment is thereby strengthened.

The true ctenolium is limited to the right valve of all extant species thus far recognized in the family Pectinidae with the exception of two species in the genus *Juxtamusium* Iredale, 1939. Members of these species also develop an identical structure on the left valve, suggesting that they have a unique mode of attachment with byssal threads extending from both the right and left sides of the byssal gape.

The only structures resembling a ctenolium in the other extant families of the Pectinacea are two types of pseudoctenolium, one formed as a row of spines on the external surface of the disk along the suture, the other forming as a series of projections from the margin of the auricle in the byssal fasciole.

Only the latter seems to have a function similar to that of the true ctenolium, but it can form only one active tooth at a time. Thread separation is therefore minimal.

Among the families in the Pectinacea, the true ctenolium is limited to the family Pectinidae and apparently has been so limited since its origin in the Mesozoic era, possibly as early as the Triassic. The structure is absent in species of the Propeamussiidae and Syncyclonemidae even though the majority of these are probably byssate throughout life. The ctenolium is also absent in young *Spondylus*, which are probably byssate before becoming permanently cemented.

In the analysis of phyletic relationships, the ctenolium can be viewed as a derived character state, the primitive state being the byssal notch without ctenolium. The ctenolium-like structures referred to herein as pseudoctenolia are analogous but not homologous. Because all known extant species of the Pectinidae have a true ctenolium, they can be viewed as a monophyletic unit, a conclusion already advanced on the basis of other congruent features of the shell and body (Waller, 1978).

Like shell microstructure, the ctenolium appears to have contributed to the success of the Pectinidae by diversifying in shallow water in spite of increasing predation pressure since the beginning of the Mesozoic era. The Propeamussiidae, lacking a ctenolium and also hindered by a sculpture-dampening shell microstructure, are diverse only in deeper water, the shallow-water species being small and cryptic in habit in tropical regions where predation pressure is strongest. The Syncyclonemidae, also lacking a ctenolium, have been low in diversity throughout the Cenozoic; at present the family is represented by only two species that are disjunct in distribution and apparently cryptic in living habit. The non-ctenoliate Spondylidae, moderately diverse in shallow water, have achieved strong attachment by becoming cemented at a very early growth stage and never evolved a ctenolium.

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LITERATURE CITED

BAVAY, A., 1902, Note sur quelques espèces du genre *Pecten*, nouvelles ou mal connues. *Journal de Conchyliologie*, 50: 399–406, pl. 8.

CADDY, J. F., 1972, Progressive loss of byssus attachment with size in the sea scallop, *Placopecten magellanicus* (Gmelin). *Journal of Experimental Marine Biology and Ecology,* 9: 179–190.

CLARK II, G. R., 1974, Calcification on an unstable substrate: marginal growth in the mollusk *Pecten diegensis. Science,* 183: 968–970.

DALL, W. H., 1895, Contributions to the Tertiary fauna of Florida, with especial reference to the Miocene Silex-beds of Tampa and the Pliocene beds of the Caloosahatchie River. Part III. A new classification of the Pelecypoda. *Transactions of the Wagner Free Institute of Science of Philadelphia*, 3(3): 479–570.

DALL, W. H., 1898, Contributions to the Tertiary fauna of Florida with especial reference to the Miocene Silex beds of Tampa and the Pliocene beds of the Caloosahatchie River. Part IV. I. Prionodesmacea: *Nucula* to *Julia*, II. Teleodesmacea: *Teredo* to *Ervilia. Transactions of the Wagner Free Institute of Science of Philadelphia*, 3(4): 571–974, pl. 23–35.

DHONDT, A. V., 1973, Systematic revision of the Chlamydinae (Pectinidae, Bivalvia, Mollusca) of the European Cretaceous. Part 2: *Lyropecten. Bulletin de l'Institut royal des Sciences naturelles de Belgique,* 48(7): 1–81, 3 pl.

GRUFFYDD, L. D., BUDIMAN, A. & NOTT, J. A., 1979, The ultrastructure of the byssus and the byssus glands in *Chlamys varia* L. (Lamellibranchia). *Journal of the Marine Biological Association of the United Kingdom,* 59: 597–603, 2 pl.

HAYAMI, I., 1973, Discontinuous variation in an evolutionary species, *Cryptopecten vesiculosus*, from Japan. *Journal of Paleontology*, 47: 301–420, 2 pl.

HERTLEIN, L. G., 1969, *Entolium* Meek, 1865. *In* MOORE, R. C., (ed.), *Treatise on Invertebrate Paleontology,* Part N, vol. 1, Mollusca 6, Bivalvia: 346–347.

KIER, P. M., GRANT, R. E. & YOCHELSON, E. L., 1965, Whitening fossils. *In* KUMMEL, B. & RAUP, D., (ed.), *Handbook of Paleontological Techniques*, Freeman, San Francisco: 453–456.

KNUDSEN, J., 1970, The systematics and biology of abyssal and hadal Bivalvia. *Galathea Report,* 11: 1–241, 20 pl.

NEWELL, N. D., 1969, Family Aviculopectinidae Meek and Hayden, 1864. *In* MOORE, R. C. (ed.), *Treatise on Invertebrate Paleontology,* Part N, vol. 1, Mollusca 6, Bivalvia: 335–341.

STANLEY, S. M., 1970, Relation of shell form to life habits of the Bivalvia (Mollusca). *Geological Society of America Memoir* 125: 1–296.

TAYLOR, J. D., KENNEDY, W. J. & HALL, A., 1969, The shell structure and mineralogy of the Bivalvia. Introduction. Nuculacea—Trigonacea. *Bulletin of the British Museum (Natural History). Zoology.* Supplement 3: 1–125, 29 pl.

VERMEIJ, G. J., 1978, *Biogeography and adaptation: patterns of marine life.* Harvard University Press, Cambridge, Massachusetts, xi + 332 p.

WALLER, T. R., 1972a, The Pectinidae (Mollusca: Bivalvia) of Eniwetok Atoll, Marshall Islands. *Veliger*, 14: 221–264.

WALLER, T. R., 1972b, The functional significance of some shell microstructures in the Pectinacea (Mollusca: Bivalvia). *International Geological Congress, 24th Session, Montreal, Canada,* 7 (Paleontology): 48–56.

WALLER, T. R., 1978, Morphology, morphoclines and a new classification of the Pteriomorphia (Mollusca: Bivalvia). *Philosophical Transactions of the Royal Society of London,* ser. B, 284: 345–365.

WALLER, T. R., 1980, Scanning electron microscopy of shell and mantle in the order Arcoida (Mollusca: Bivalvia). *Smithsonian Contributions to Zoology,* 313: 1–58.

YONGE, C. M., 1967, Observations on *Pedum spondyloideum* (Chemnitz) Gmelin, a scallop associated with reef-building corals. *Proceedings of the Malacological Society of London,* 37: 311–323.

NOTE ADDED IN PROOF

Research on the phylogenetic relationships of pectinacean taxa in the two and one-half years since this paper was submitted for publication corroborates the conclusions herein but also necessitates nomenclatural changes.

A few well-preserved specimens of *Pleuronectites laevigatus* von Schlotheim, 1820, type-species of *Pleuronectites* from the Triassic of Europe, were examined at the University of Paris-South, Orsay, France. These appear to have a pectinacean-type ligament system, not the aviculopectinacean type, the confusion apparently stemming from the ligament system migrating ventrally with growth as in other attached Pectinacea, thus leaving a ligament area that superficially resembles that of aviculopectinaceans. This ctenoliate genus is, therefore, the earliest known member of the family Pectinidae.

No ctenolium could be found even in the very early growth stages of well-preserved specimens of *Entolium* from the Jurassic and Cretaceous of Europe, and this genus is clearly a member of the non-ctenoliate clade. Examination of many taxa related to *Entolium* and *Syncyclonema* indicated that the family Syncyclonemidae, which I introduced in 1978, should be reduced in rank to a level within the family Entoliidae von Teppner, 1922. Specimens of "*Syncyclonema*" from the Mesozoic indicate that the living species assigned to *Syncyclonema* above are end members of a group which has existed since early in the Mesozoic and which is generically distinct from type *Syncyclonema* of the Cretaceous. This means that the living species and their fossil ancestors should be returned to *Pectinella* Verrill, 1897, a genus within the family Entoliidae and within the subgroup containing *Syncyclonema.* The relationships among these and other supraspecific taxa in the families Propeamussiidae, Entoliidae, Neitheidae, Spondylidae, and Pectinidae, and among the families themselves, will be examined in detail in a monograph in preparation.

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ASPECTS OF THE FUNCTIONAL MORPHOLOGY OF SOME FRESH-WATER BIVALVE NERVOUS SYSTEMS: EFFECTS ON REPRODUCTIVE PROCESSES AND ADAPTATION OF SENSORY MECHANISMS IN THE SPHAERIACEA AND UNIONACEA

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ABSTRACT

The soft bodies of mollusks provide the animals with a neuroanatomical context within which the plasticity and variability of the molluscan nervous system can be exploited. For the higher taxa of fresh-water bivalves, functional morphology not only of the reproductive systems (in the tradition of Ortmann, 1911), but also of nervous systems and sensors and effectors can account for much adaptive radiation of fresh-water bivalves in stable and in rapidly changing habitats.

Histological/neuroanatomical and behavioral evidence is presented here concerning several neural entities for (1) the hermaphroditic sphaeriacean bivalve *Corbicula fluminea*; and (2) several dioecious unionacean bivalves, genera *Lampsilis* and *Carunculina*. For *C. fluminea*, details of (a) gonoduct innervation; (b) development of "follicular ganglia" along with maturation of oogenic and spermatogenic follicles; and (c) peculiar, conjoined statocysts—are evaluated in their neuroanatomical context. For *Lampsilis*, details of (a) mantle ganglia organization and (b) separate, cislaterally innervated statocysts; and for *Carunculina*, innervation and behavior of the "thumb-twiddling" caruncles—are similarly evaluated.

The foregoing tend to corroborate and amplify characteristics of the reproductive process which distinguish the sphaeriacean and unionacean bivalves. Nervous systems of mollusks do frequently have clusters of neuronal soma at the periphery of even very small nerves, and some of these clusters can be demonstrated (as in the present study) to be associated with peculiar reproductive or locomotor behavior. It seems, therefore, that further investigations of molluscan nervous systems will provide important clues to the environmental past and phylogenetic history of these organisms and also to their adaptational future.

Key words: behavior; bivalve; gametogenesis; ganglia; nerves; reproduction.

For the fresh-water taxa of the class Bivalvia, a number of investigators, including Ortmann (1911), Walker (1917), van der Schalie (1952), Fretter & Graham (1964), Heard & Guckert (1970), Johnson (1970), Clarke (1973) and Burch (1973) have urged the use of anatomical characteristics of reproductive processes to separate/distinguish taxa. Davis & Fuller's (1981) immunological study lends support. In a different context, Hunter (1964, p. 90) notes ". . . bivalves of fresh waters show little of the adaptive radiation that gives particular interest to functional morphology in most groups of marine bivalves. The four major fresh-water families (i.e. Unionidae, Mutelidae, Corbiculidae, Sphaeriidae) are remarkably uniform in structure. . . ." I agree with the former workers and disagree with the latter worker on the basis of evidence and argument which follow.

In this paper, I will provide evidence from which I will argue that at least for the higher taxa of fresh-water bivalves, functional morphology not only of reproductive systems, in the tradition of Ortmann (1911), but also of *nervous* systems and sensors and effectors can account for much adaptive radiation of fresh-water bivalves in stable and in rapidly changing habitats. I will review findings from some of my studies of the morphology and behavior of the sphaeriacean bivalves, *Corbicula* cf. *C. fluminea* (Müller, 1774), and of certain unionacean bivalves, especially members of the genera *Lampsilis* and *Carunculina*. Emphasis will be on analyses of certain peripheral neural entities. I also will summarize findings which provide neuroanatomical context for the former entities, and which together relate to the bivalves' reproductive, spawning or locomotor capabilities. Finally, I will argue that functional anatomy studies of such peripheral neural elements in

bivalves are important not only in interpreting systematic position of these animals, but also in evaluating their capabilities for future adaptation.

METHODS AND MATERIALS

Living *Corbicula fluminea* used for this study were from the Arkansas River near Russellvill, Pope County, Arkansas, and from the Buffalo River in Madison County, Arkansas. *Lampsilis* spp. described here include those obtained as detailed in Kraemer (1970). *Carunculina texasensis* (Lea, 1857) specimens were obtained by Bob West (Arkansas Power and Light Co.) from Rock Creek (tributary of the Arkansas River in Pulaski county, Arkansas), maintained in aquaria and observed manifesting their peculiar spawning behavior in June 1981. Observations of their behavior are reported below in detail for two reasons: (1) no previous record has been found to exist in the literature; and (2) opportunity for making such observations becomes increasingly rare as populations of these animals disappear from heavily managed rivers of the United States. Preserved specimens of *C. texasensis* collected in Nueces River in Texas in August 1978 were provided by Mark Gordon (University of Arkansas); preserved specimens of *Carunculina glans* (Lea, 1834) were collected from the Illinois River in Washington County, Arkansas in October 1964.

Histological material was prepared as described elsewhere (Kraemer and Lott, 1977). Photomicrographs were made with a Leitz Ortholux microscope equipped with a 35-mm Leica camera, and with a Wild M5 Stereomicroscope in conjunction with a 35-mm Wild Mka 1 camera.

LIST OF ABBREVIATIONS

A	anus
AS	anal or exhalant siphon
BRS	branchial shelf, membrane which separates exhalant from inhalant or branchial chamber
BS	branchial or inhalant siphon
C	caruncle
CO	connective tissue capsule
CT	connecting tube between left and right statocysts of *Corbicula fluminea*
CVC	cerebro-visceral connective
DO	site of distal *pore* which appears in each ovisac of the marsupial gills near the culmination of mantle flapping/ spawning behavior
E	"eyespot" of mature female *Lampsilis* mantle flap
EM	cross-section of embryo within the gonoduct of *C. fluminea*
F	foot
G	gill
GL	gonoduct/gonopore lip
GO	gonoduct
IG	inner gill
L	lumen
LBN	left branchial nerve
LC	left caruncle
LMF	left mantle flap of mature female *Lampsilis*
LMG	left mantle ganglion of mature female *Lampsilis*
LOG	left outer gill
LP	labial palp
LPN	left pallial nerve trunk
LV	left shell valve
M	posterior portion of left and right outer gills which are differentiated into marsupia for glochidia larvae
NC	nerve from cerebral ganglion innervating follicular "ganglion"
NF	nerve fibers
NP	neuropile-like aggregation of nerve fibers within the follicular "ganglion"
O	ovisac of gravid marsupial gill, charged with glochidia larvae
P	papilla
PA	posterior adductor muscle
RC	right caruncle
RM	right mantle lobe
RMF	right mantle flap of mature female *Lampsilis*
RPN	right pallial nerve trunk
RV	right shell valve
S	neuronal soma
SB	suprabranchial chamber
SE	sensory epithelium
SF	spermatogenic follicle
SL	statolith
SM	clump of mature sperm of sperm "morula"
SN	statocyst nerve
T	"tail" of mature female *Lampsilis* mantle flap
VG	visceral ganglion
VM	visceral mass

OBSERVATIONS OF NEURONAL STRUCTURES

A. Dioecious unionaceans (Fig. 1)

1. The mantle ganglia of female *Lampsilis ventricosa* (Barnes, 1823)

L. ventricosa is one of a number of indigenous fresh-water mussels which have developed a unique spawning apparatus and spawning behavior complex characteristic only of the mature gravid females of this dioecious group (Kraemer, 1970). In the mature gravid female, the water tubes of the posterior portion of each outer gill are filled with fully differentiated glochidia. Upending herself in the substrate, the mussel extends a pair of mantle flaps equipped with "eyespots" and "tails" from between the posterior margins of her shell valves. Charged marsupia are typically then protruded between the flaps. The mantle flaps are moved rapidly in *L. ventricosa*—up to three times per second—and to the human observer resemble a small swimming fish. Mantle flap movements are paired pulses which are initiated near the tail ends of the flaps and travel postero-dorsally toward the eyespot ends (Fig. 2). After several weeks of flap movements glochidia are discharged. The foregoing phenomena, as well as species variations (in which some

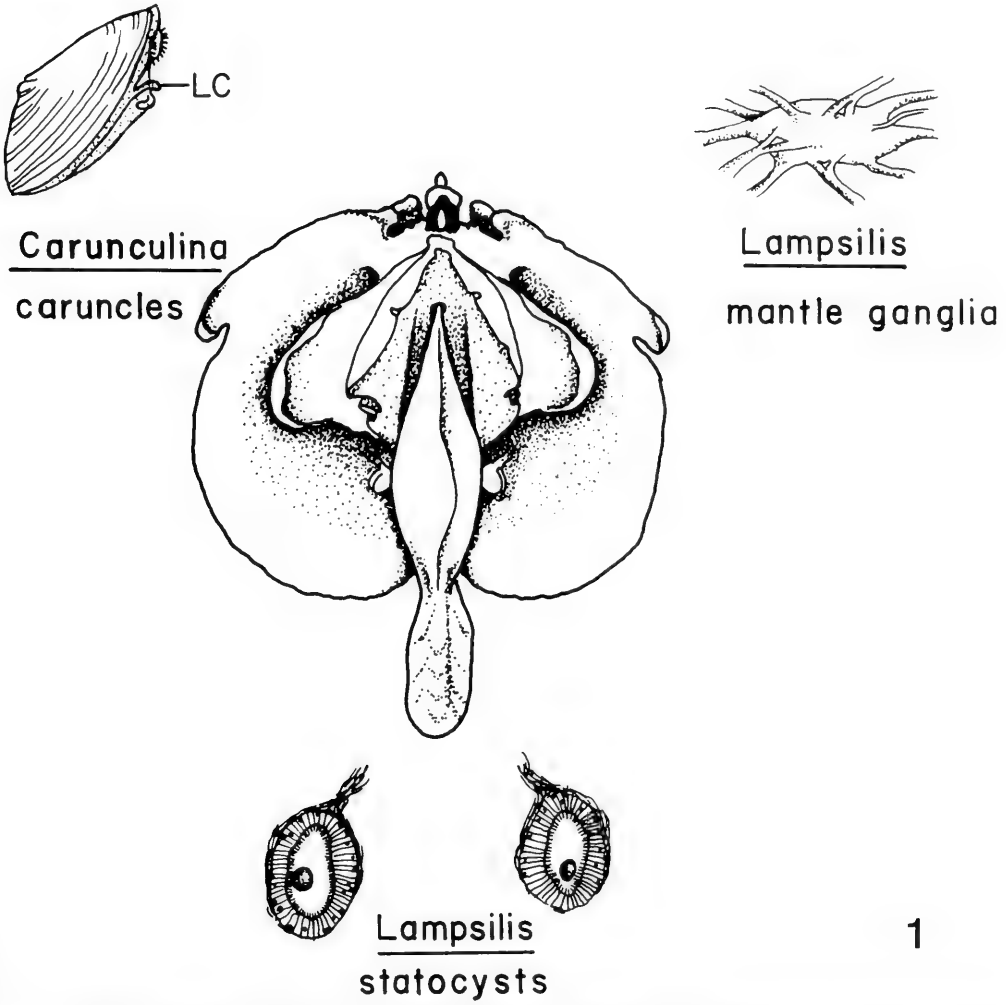


FIG. 1. Summary diagram indicating the peripheral neural entities of the unionacean bivalves *Lampsilis ventricosa* and *Carunculina texasensis*, discussed in the section of "Observations."

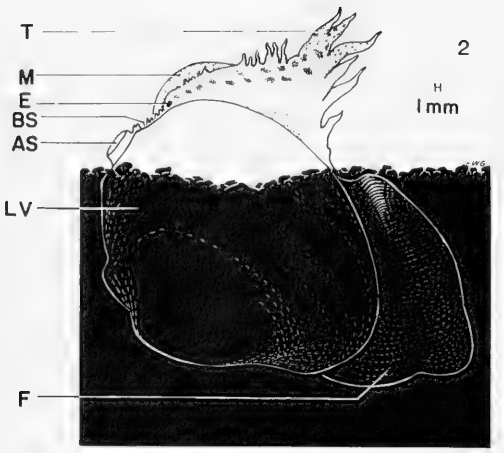


FIG. 2. Diagram of female *Lampsilis fasciola* during flapping behavior. Mantle flaps are protruded between the valves and pulsing mantle flap movements proceed from "tail" (T) to eyespot (E) end of flap. Posterior end of a gravid outer gill or marsupium (M) protrudes between flaps.

flaps and flap movements are *not* "fish-like") found in *Lampsilis radiata siliquoidea* (Barnes, 1823), *Lampsilis fasciola* (Barnes, 1834), *Lampsilis brevicula brittsi* (Simpson, 1900), have been detailed elsewhere (Kraemer, 1970, 1974).[1]

If the animal is completely removed from its shell valves and placed on its dorsal surface for dissection, one notes that the posterior mantle lobe edges are unfused (Fig. 3A). How then does the animal co-ordinate the movement of both flaps? Dissection in the area of the visceral ganglion and of its peripheral pallial nerves reveals the presence of a pair of mantle ganglia in the mature female (Fig. 4A, 5A). Histological examination (Fig. 5B) verifies their ganglionic organization: a sheath of connective tissue enclosing a peripheral layer of neuronal soma, the latter surrounding a central neuropil. Similar mantle ganglia were not found in neuroanatomical dissections of mature specimens of male *L. ventricosa* (Fig. 4B), or in *Amblema plicata* and *Anodonta imbecilis*. They were not included in previously published figures of peripheral bivalve neuroanatomy (Duvernoy, 1852; Rawitz, 1887; Bullock & Horridge, 1965).

The mantle ganglia seem to be characteristic only of the mature female lampsilid mussel and their location is suggestive of association with mantle flap movements. Each is positioned in the mantle tissue near the "tail" end of a mantle flap, the point at which each mantle flap movement begins. It is possible that these mantle ganglia are the pacemakers for the mantle flap movements, or that they function to co-ordinate the mantle flap movements which are mediated by the visceral ganglion.

2. The "caruncles" of *Carunculina*

Carunculina parva is a hermaphroditic species (van der Schalie, 1970) which belongs to the same subfamily (Lampsilinae) as *Lampsilis*. In the mature animals a conspicuous pair of "caruncles" is developed, one on either side of the inner surface of the

[1]Davis & Fuller's (1981) article on genetic relationships among recent Unionacea of North America has just recently appeared. Davis and Fuller carried out an impressive immunological study of 52 unionacean species from 27 genera. They also assembled a thoughtful evaluation of noteworthy systems of unionacean classification against which to compare the evidence from their cladogram. With some of their extrapolations to functional morphology, however, I cannot agree. One sample (: 244) follows:

> "For example, the probasal margins of *Lampsilis* are piscine in character; the implication is that predatory . . . fish species will attack the 'prey' represented by the mussel's mantle margins and will be showered with glochidia *if*, as is often true in the case of heterogeneous genera, discharge of parasitic larvae is through the marsupial wall and not through the excurrent mantle aperture."

After several years of careful study, Kraemer (1970) elucidated the flapping behavior complex in living *Lampsilis* spp. and reported in detail from first hand knowledge on many aspects of the matter, including: (1) The probasal margins of *Lampsilis* spp. are not necessarily "piscine" in character, either in structure or function. (2) *No* evidence was found of fish "attacking" the mantle flaps. To the contrary, much evidence was accumulated that in stream environments mantle flap movements *cease* when fish appear in the vicinity of the moving flaps. (3) *No* evidence was ever found of glochidia being released in a "shower" from the ovisacs of the marsupia. Occasionally distal margins of marsupial ovisacs ruptured, and the glochidia from an ovisac would consequently be aborted, not as a "shower" but as a lump, a conglutinant. (4) The "*if*" in the Davis and Fuller sentence quoted above is ironic. Kraemer (*ibid.*) reports specifically that a tiny hole is seen to appear in the distal edge of each ovisac near the end of the flapping behavior complex. Further she reports that glochidia in a series of monitored ovisacs were seen to disappear from those ovisacs over a 12-h period. All circumstantial evidence indicated that the avenue for egress for glochidia was from the several holes described, and indeed not as an aborted conglutinant, *not* as a "shower," and most certainly not from the excurrent siphon.

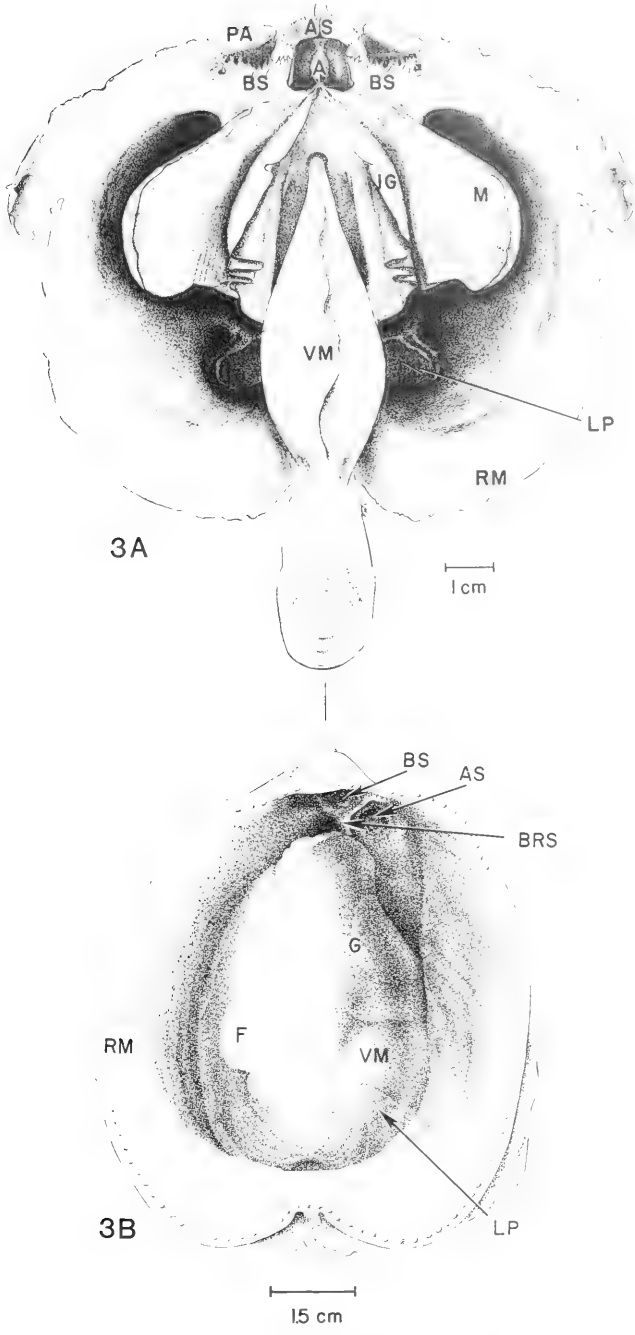


FIG. 3A. *Lampsilis ventricosa* (Barnes). Drawing of relaxed, preserved mature female specimen removed from its shell valves, placed on its dorsal surface and viewed from the rear. Note flaring mantle lobes, which show almost no fusion. Compare with B. From Kraemer, 1979. B. *Corbicula fluminea* (Müller). Drawing of relaxed fresh mature specimen removed from its shell valves, placed on its dorsal surface and viewed from the rear. Note thickened, fused posterior portion of mantle lobes, and narrowed pedal gape. Compare with A.

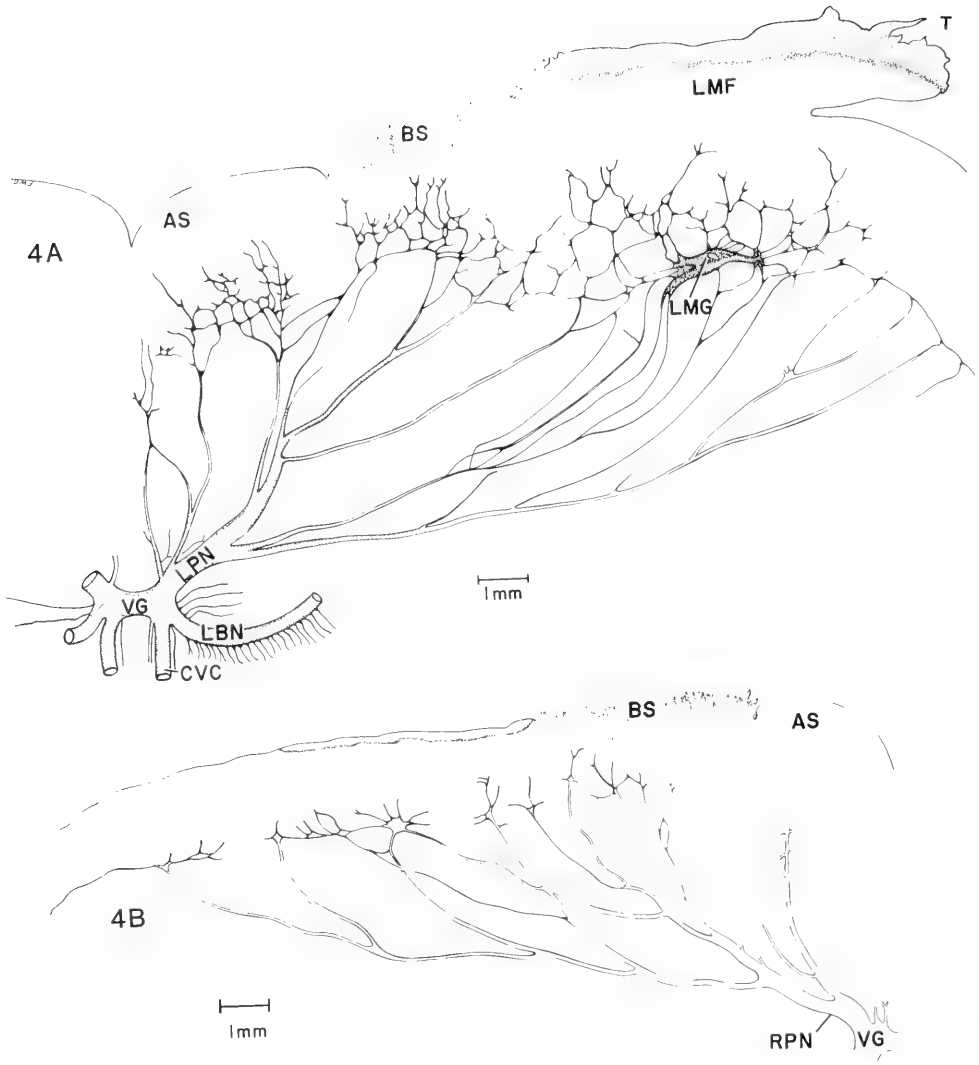


FIG. 4A. Drawing of dissection of posterior region of mantle of mature female *Lampsilis ventricosa*, showing visceral ganglion (VG), innervation of left rear mantle lobe and location of accessory mantle ganglion (LMG), adjacent to mantle flap "tail" (T). B. Drawing of dissection of posterior region of mantle of mature male *Lampsilis ventricosa*, showing visceral ganglion (BV) and innervation of right rear mantle lobe.

mantle margin, and anteroventrad to the branchial siphon (Simpson, 1914).

Prior to this study, I examined more than 25 specimens of *C. parva* to determine the locations of the "caruncles." Neuroanatomical dissections were made of four specimens. Distribution of posterior pallial nerves is shown in Fig. 7. No evidence of mantle ganglia near the "caruncles" was found.

In June 1981, two mature gravid individuals of the apparently dioecious species *Caruncu-lina texasensis* were observed intermittently for more than 5 h, as they showed the behavior described below. Bob West verified that the animals had been showing similar behavior for about two weeks. (Mantle edges of female *C. texasensis* are shown in Fig. 6.)

(a) *"Pulsing" behavior*: Lying on one valve or the other, or lying hinge down in the substrate, both mussels showed bouts of pulsing behavior which continued for 15 min, 30 min, 60 min, or more.

"Pulsing" behavior is the term I am applying to the following: In opposing short pigmented

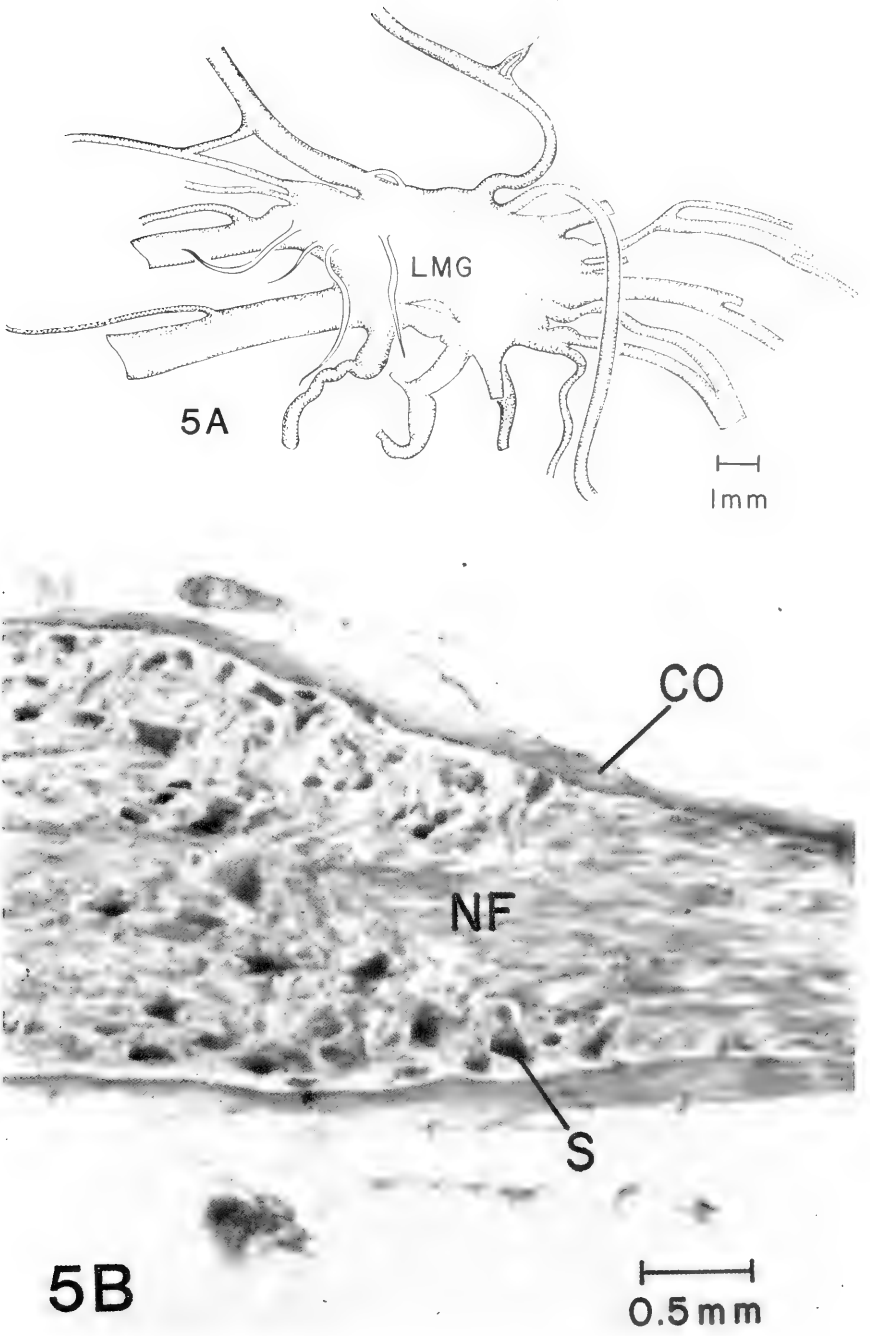


FIG. 5A. Drawing of accessory mantle ganglion, removed from left mantle lobe of specimen of *Lampsilis ventricosa*, shown in Fig. 3A. B. Sagittal section of mantle ganglion removed from rear left mantle lobe near "tail" of mantle flap of mature female specimen of *Lampsilis ventricosa*.

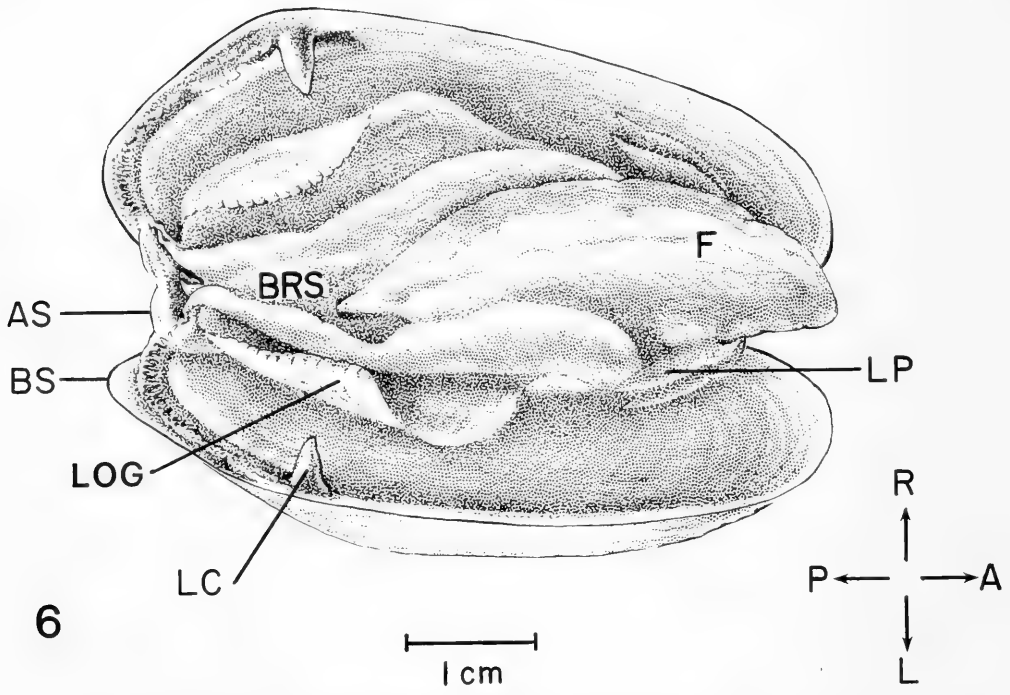


FIG. 6. Drawing of preserved mature female *Carunculina texasensis* to show left (LC) and right caruncles on inner surface of mantle antero-ventral to branchial siphon.

sectors of the inner lobe of each mantle edge, immediately adjacent to the branchial siphon, paired movements began as synchronous pulses and passed quickly along the mantle edges to the region of the two caruncles. The pulses were rapid, about 3/sec. They were followed by a one- or two-second pause, then repeated. Pulses occurred typically in groups of three, but often varied up to seven or more. As the paired pulsing movements passed along the thin membranous flanges of the inner mantle lobes, round, white, bead-like distal edges of the ovisacs of the gravid marsupial gill of the animal could be seen exposed between the lobes. The effect was a kind of rippling "smile" of the mantle edges.

During the few hours available for observation, other pulses were seen (in the mantle lobe areas) which moved in the opposite direction, from caruncle region to branchial siphon. These occurred less frequently and their relation to the "pulsing" behavior described above could not be determined.

(b) *Caruncular behavior*: Following a course of pulsing behavior, first one "caruncle" and then the other extended from between the mantle lobes. The caruncles were white, completely unpigmented and elongate, with very much the *shape* of "thumbs." That is, each caruncle was somewhat splayed distally, tapered, and cylindrical near its point of attachment on the inner surface of the mantle lobe.

The first thumb-like caruncle to emerge was observed to move in a rotary plane at right angles to the longitudinal axis of the animal. When the second caruncle emerged, both caruncles rotated, frequently touching each other at their distal "thumb" tips. Viewed from the side through the aquarium glass, one caruncle rotated clockwise about its base, the other counter-clockwise (Fig. 8A, 8B, 8C). From time to time, the thumb-like caruncles would reverse their respective rotary movements. The rotary caruncular movements continued at an even rate of about 1/sec for 15 min or more. The longest sequence observed lasted 1 h. In several instances caruncular movements ceased as the caruncles were withdrawn and the paired pulsing movements of the mantle edges resumed.

When the animal lay on its dorsal surface in the substrate, and its moving caruncles were viewed from a postero-ventral aspect, the

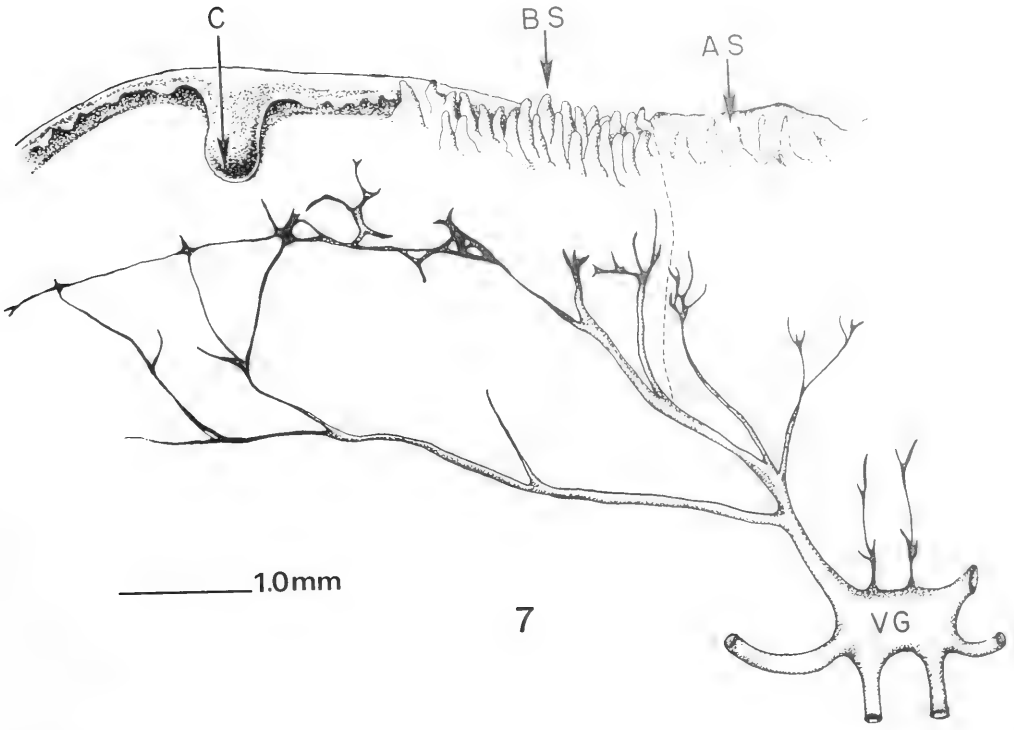


FIG. 7. Drawing of posterior region of mantle of *Carunculina parva*, showing the visceral ganglion (VG), innervation of right rear mantle lobe and location of "caruncle" (C).

caruncles resembled a pair of opposing, twiddling thumbs (Fig. 8D). J. P. E. Morrison (personal communication) had used the expression "thumb twiddling" years ago in commenting on the movement of *Carunculina* caruncles. Until the observations described above, however, I had neither seen, nor encountered a detailed description of such movement. Now it is obvious to me that Morrison's characterization of "thumb twiddling" is apt for the caruncular movements.

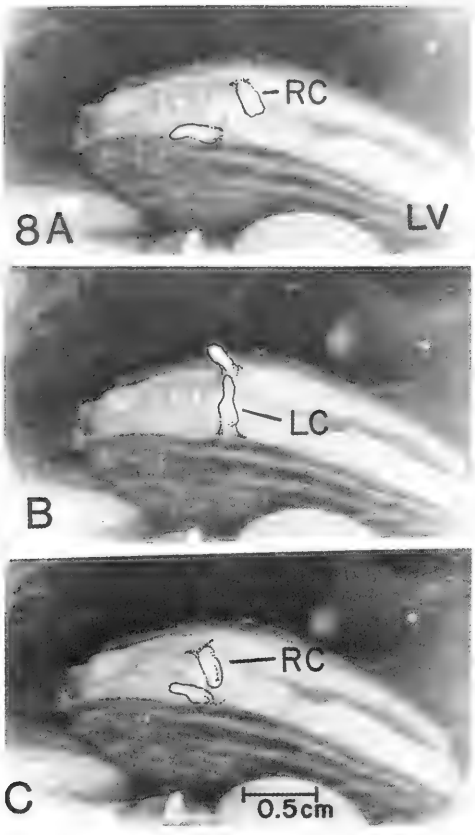
It seems likely that the pulsing movements and caruncular movements of *Carunculina* mantle edges have a spawning function similar to the mantle flap behavior complex of *Lampsilis* (Kraemer, 1970), although the movements of *Carunculina* are differently initiated and displayed. It seems possible that peripheral aggregation of neuronal soma may be found where pulsing movements are initiated, near the ventral edges of the branchial siphon.

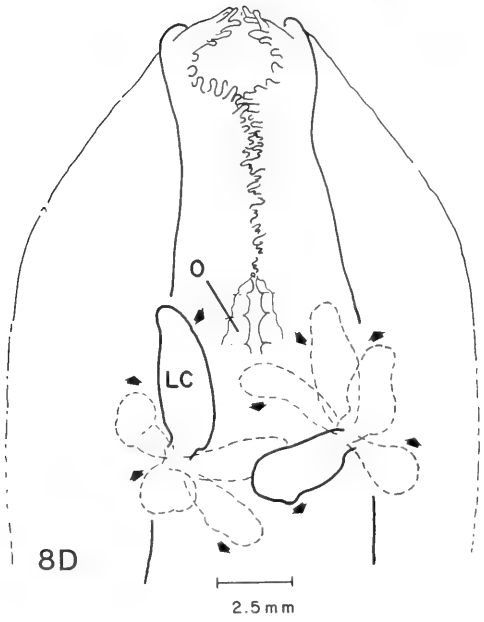
3. Statocysts of *Lampsilis ventricosa*

As reported earlier (Kraemer, 1978a) each of the paired statocysts is a small translucent globe (about 2 mm in diameter in an animal 12 cm long). The two statocysts are widely separated from each other. Each is embedded deep in the latero-mid-posterior portion of the foot. Each statocyst is attached to a statocyst nerve which branches from the cis-lateral cerebro-pedal connective. The statocyst nerve penetrates the connective tissue capsule of the organ, and branches among the tall ciliated columnar epithelial cells which line the capsule (Fig. 9A, 10A). The central cavity of the statocyst contains one or more hard statoliths.

Slow stepping movements of the foot of *Lampsilis* during normal locomotion may be responding to information from the distally placed statocysts.

During the flapping behavior complex





which accompanies spawning of glochidia in *L. ventricosa*, the female typically assumes a prolonged "headstand," in which the foot functions as a vital prop (Kraemer, 1970). In this instance too, *L. ventricosa* statocysts are well suited to aid the animal in maintaining its spawning "stance."

B. Monoecious sphaeriaceans (Fig. 11)

1. Innervation of the gonoduct "lips" in *Corbicula fluminea*

Even before there is any histological evidence of gamete or gonad development in young clams (2–3 mm long), the lips of the two gonoducts are well differentiated (Kraemer, 1978b). Not until the clam has undergone further development of its gonads (4–6 mm specimens), however, does one see extensive innervation of the gonoduct lips. Many nerve fibers branch directly from each cislateral cerebro-visceral connective as it emerges from the visceral mass at the level of each gonoduct opening (Fig. 12A).

2. Follicular ganglia of *Corbicula fluminea*

Oocyte development begins in small animals (4–6 mm long) when gametocytes differentiate in close association with the basement membranes of the gut and the digestive glands. A developmental sequence may be traced in which slender oogenic follicles elongate and ramify through the visceral stroma; the follicles later become crowded with enlarged oocytes; the oocytes subsequently appear stalked. Eventually, the oogenic follicles appear to be mostly empty of oocytes, and also may contain what appear to be young embryos (Fig. 12B). Not until the oogenic follicles are well developed is there histological differentiation of spermatogenic follicles (Kraemer, 1978b). Spermatogenic follicles are never more than one-fourth as numerous as the oogenic follicles and are located mostly peripheral to the oogenic follicles.

FIG. 8. Sequence (A, B, C) of photographs showing caruncular movements in *Carunculina texasensis,* taken through aquarium glass, June 11, 1981. Animal is lying on its left shell valve. Note that right caruncle (RC) is showing counter-clockwise movement and left caruncle (LC) is showing clockwise movement. D. Diagram of caruncles from dorsal aspect, illustrating "thumb twiddling" movements of the opposed caruncles.

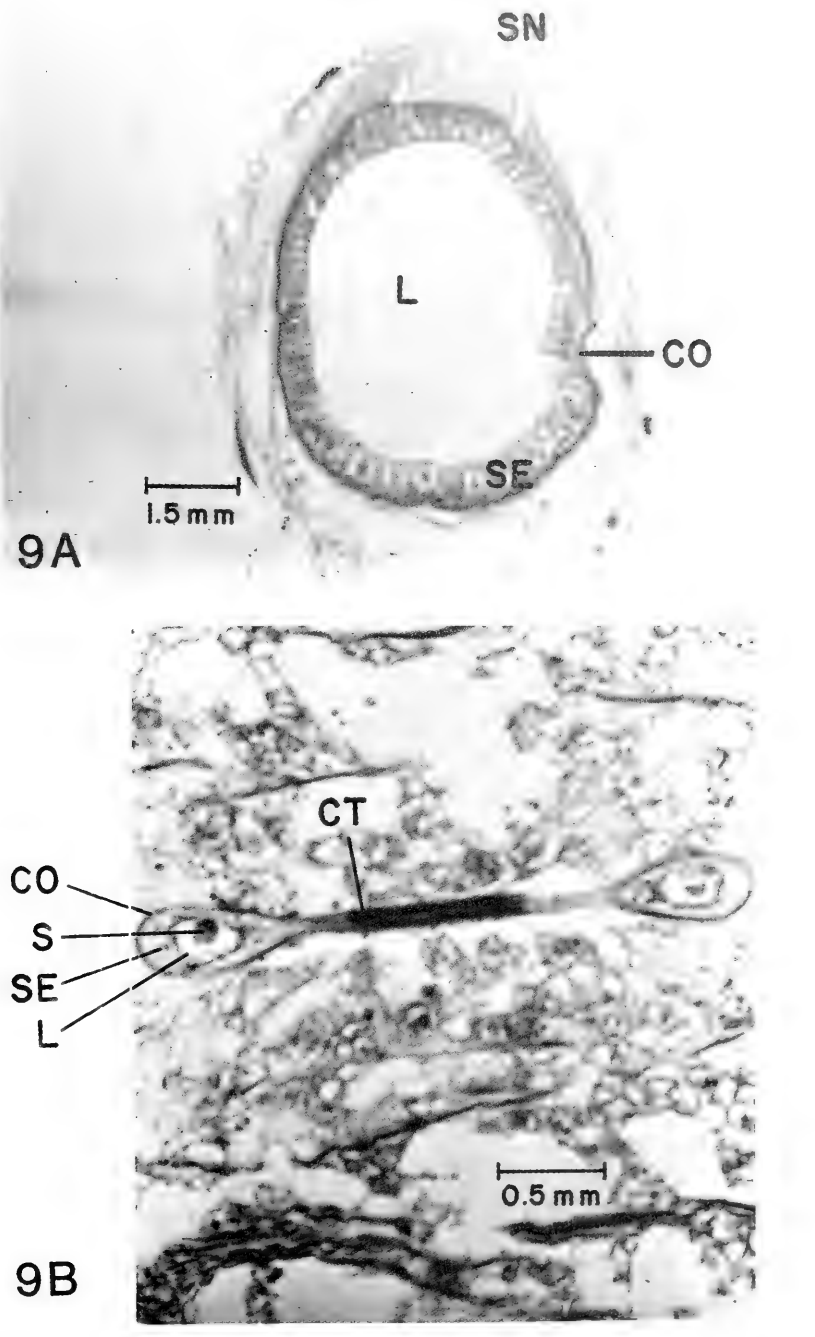
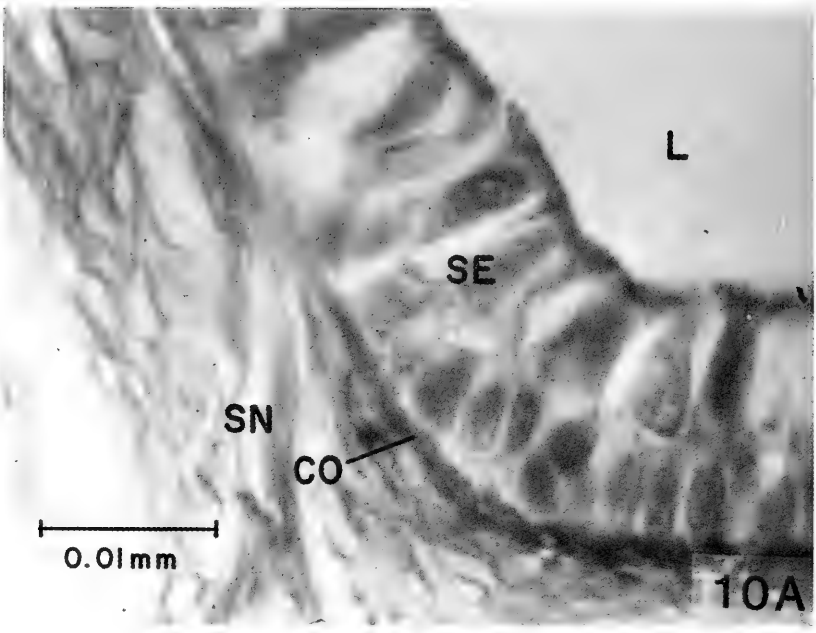


FIG. 9A. Sagittal section through left statocyst of *Lampsilis ventricosa*, showing attached statocyst nerve (SN), connective tissue capsule (CO), sensory epithelium (SE) and lumen (L). Break in the tissue on the right side of the statocyst capsule was the site through which the hard statolith "popped" during sectioning of the tissue. From Kraemer, 1978a. B. Cross-section of *Corbicula fluminea*, through region of visceral mass near pedal ganglion, showing unusual statocysts in the midline of the body, conjoined by a tube (CT). From Kraemer, 1978a.



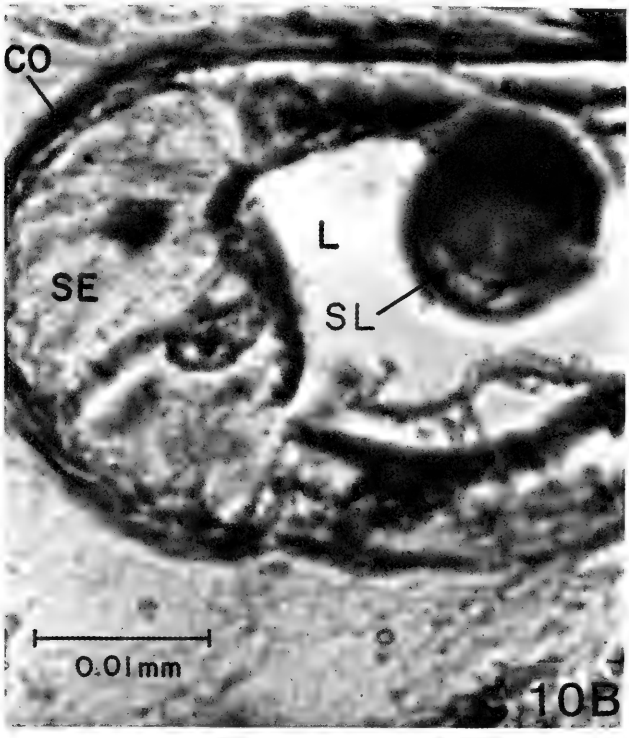


FIG. 10A. Photomicrograph of sensory epithelium (SE) of statocyst of *Lampsilis ventricosa*, showing innervation by neuronal fibers of statocyst nerve (SN). B. Photomicrograph of right statocyst of *Corbicula fluminea*, showing epithelium (SE) and statolith (SL) A, B from Kraemer, 1978b.

By the time spermatogenic, as well as oogenic follicles, are well differentiated, paired aggregation of apparent neuronal soma appear at the *confluence* of male and female follicles, usually at four locations: (1) in the postero-ventral part of the visceral mass near a large vertical loop of the intestine; (2) the mid-ventral portion of the visceral mass dorso-lateral to the pedal ganglion; (3) in the mid-anterior region of the visceral mass, postero-dorsal to each cerebral ganglion; and (4) in the mid-dorsal region of the visceral mass not far from cerebro-visceral connectives and gonoducts. I have tentatively named these clusters of neuronal soma "follicular ganglia" (Kraemer, 1978b). In addition to the apparent neuronal soma, each putative follicular ganglion was seen to enclose a dense neuropil-like core and to be innervated by slender nerves from neighboring ganglia (cerebral, pedal) (Fig. 13). A cavity of unknown function, lined with well-differentiated, ciliated cuboidal epithelium was observed in many of the follicular ganglia.

Possible function of the "follicular ganglia" may be to co-ordinate gamete production and subsequent internal and/or self-fertilization. Circumstantial evidence in support of this hy-

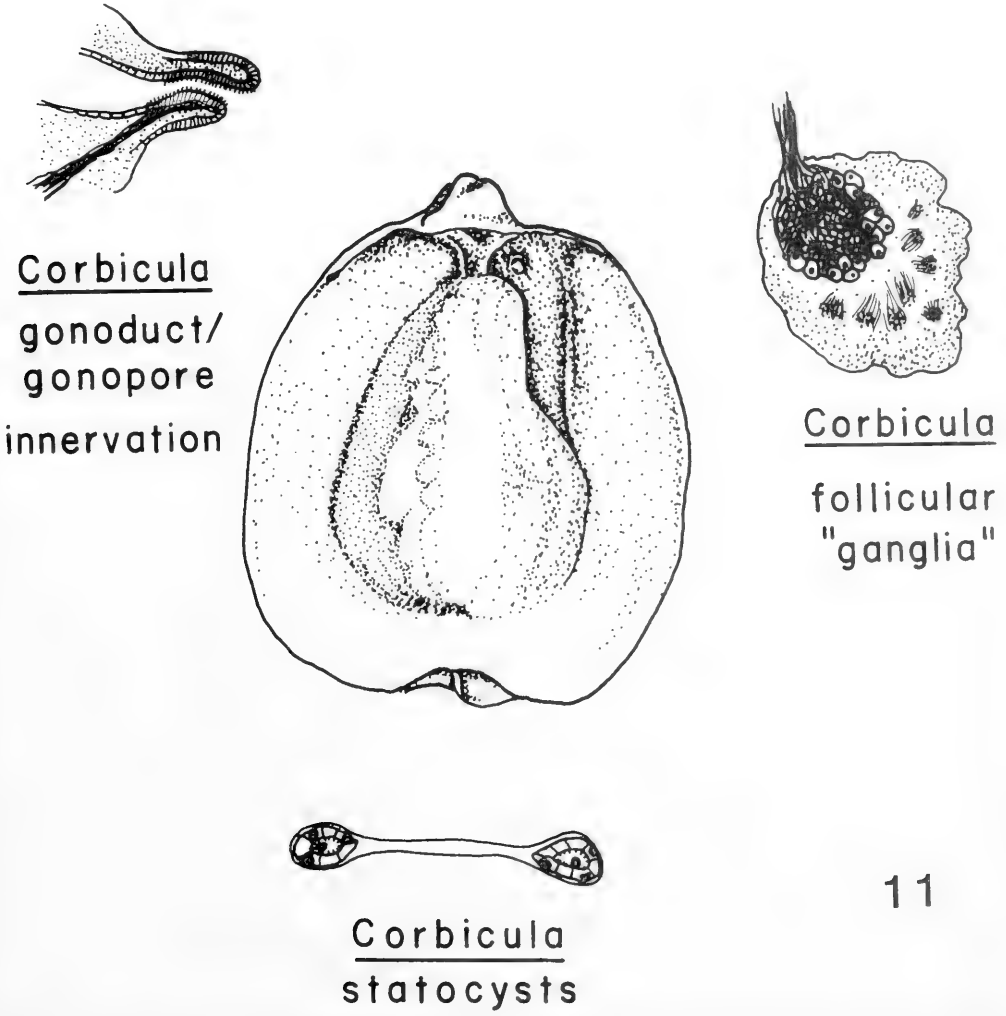


FIG. 11. Summary diagram indicating the peripheral neuronal entities of the sphaeriacean bivalve *Corbicula fluminea,* discussed in the section of "Observations."

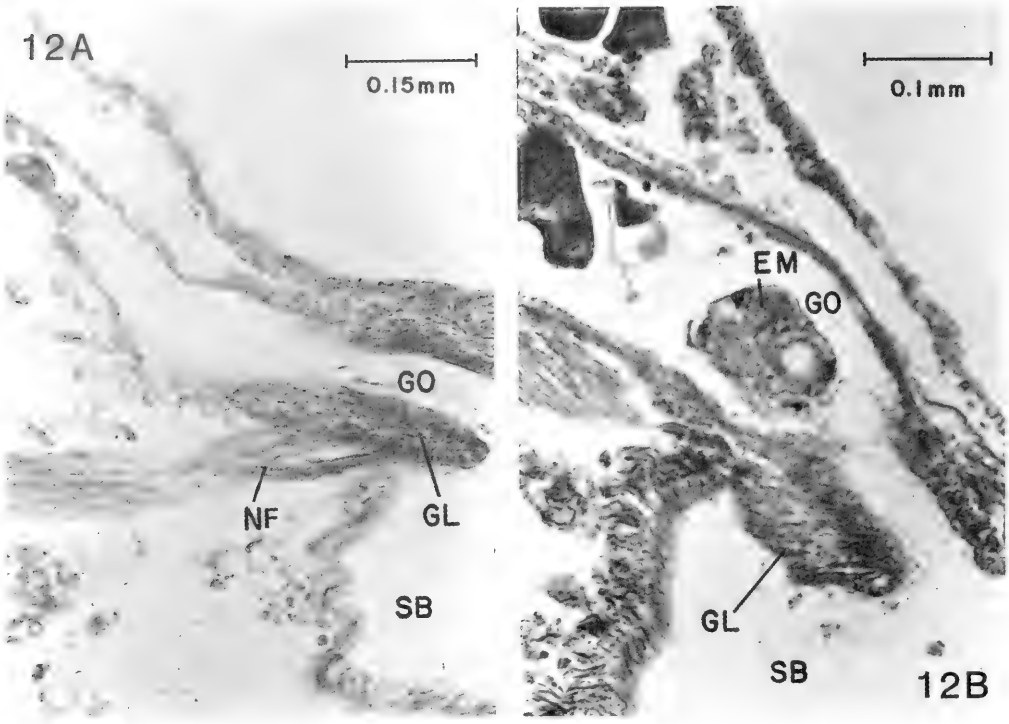


FIG. 12A. Photomicrograph of sagittal section of *Corbicula fluminea* through region of gonoduct lips (GL), showing innervation by means of neuronal fibers (NF) from cerebrovisceral connective. From Kraemer, 1978b. B. Photomicrograph of sagittal section of *Corbicula fluminea* through region of gonoduct lips (GL), showing section of embryo (DM) in the lumen of the gonoduct (GO).

pothesis includes the repeated observation of embryos within follicles near the follicular ganglia, and the subsequent appearance of embryos in the gonoduct (Fig. 12B) from where the embryos could be traced into marsupial chambers of the inner gills.

Studies on the follicular ganglia of *Corbicula fluminea* are continuing. Currently, I do not know whether, once the follicular ganglia have differentiated, they regress or are maintained by the animal.

3. Statocysts of *Corbicula fluminea*

Statocysts in this animal contrast sharply with those of *L. ventricosa* (Kraemer, 1978a). Their position in the dorsal part of the foot near its border with the visceral mass is comparable to that of *L. ventricosa.* Where the statocysts of *L. ventricosa* are displaced laterally, however, *C. fluminea* statocysts are close together in the midline of the body, and are horizontally *conjoined* by a connecting tube (Fig. 9B) The statocysts are tiny (about 100 μm in diameter in a 4-mm clam). Each is surrounded by a connective tissue capsule and lined with cuboidal epithelium (Fig. 10B). Within the cavity of each statocyst is a single large statolith. Innervation of the statocysts has not been worked out.

Conjoined by a fluid-filled tube, the statocysts of *C. fluminea* seem well suited to detecting the rapid forward-and-back, side-to-side movements which its foot manifests. As already noted (Kraemer, 1978a), it seems reasonable to hypothesize that other bivalved mollusks capable of rapid foot movements also may have conjoined statocysts.

DISCUSSION AND CONCLUSIONS

Contrasting features of the peripheral neuroanatomy of certain sphaeriacean and unionacean bivalves discussed in the following paragraphs are summarized in Table 1.

If we compare a relaxed specimen of *L. ventricosa* alongside a similar specimen of *C. fluminea* lying ventral side up, similar to the manner in which we typically view the an-

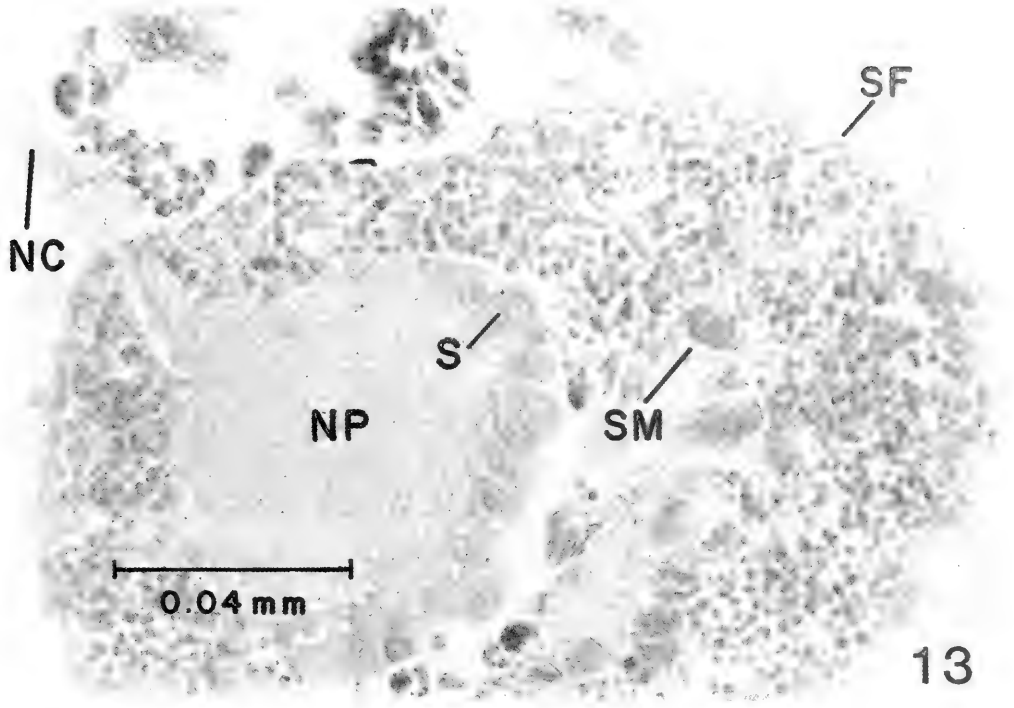


FIG. 13. "Follicular ganglion" section from anterior region of visceral mass of *Corbicula fluminea*, posterior and slightly dorsal to cerebral ganglion. Note innervation by means of nerve fibers (NC) from cerebral ganglion, many neuronal-like soma (S) at periphery of "ganglion," and its location within a well-developed spermatogenic follicle. From Kraemer, 1978b.

TABLE 1. Summary of peripheral neuronal complexes discussed in "Observation" section which account for differences in functional morphology of nervous systems in representative species of the Unionacea and Sphaeriacea, respectively.

Neural complex	Unionacea: *Lampsilis ventricosa* *Carunculina texasensis*	Sphaeriacea: *Corbicula fluminea*
Reproductive neuronal structures	In mantle: *L. ventricosa:* paired mantle ganglia in mature female near flaps antero-ventral to branchial siphon. (Figs. 4A, 5) *C. texasensis:* paired "caruncles" in mature female antero-ventral to branchial siphon. (Figs. 6, 7, 8)	In visceral mass: *C. fluminea:* development of innervation of gonoduct lips on postero-dorsal surface of visceral mass. (Fig. 12A) *C. fluminea:* development of pairs of follicular "ganglia" at confluences of oogenic with spermatogenic follicles, within visceral mass. (Fig. 13).
Statocysts	*L. ventricosa:* paired statocysts widely separated, each innervated by nerve from cislateral cerebropedal connective. (Figs. 9A, 10A)	*C. fluminea:* pair of conjoined statocysts in midline of visceral mass immediately dorsal to pedal ganglion. (Figs. 9B, 10B)

atomy of a complex bilaterally symmetrical animal (Fig. 3), it is at once evident that the former shows almost no fusion of the mantle lobes, and that the posterior mantle lobe ends are flared elaborately into the mantle flaps of the mature female. During normal siphoning and locomotor behavior, the unfused mantle lobes of *Lampsilis* spp. expose these animals to their river environment. During spawning behavior, which involves prolonged periods of the "headstand" and the flapping behavior complex (Fig. 2), the gravid female is even

more exposed generally to the water. By contrast, the fused thickened posterior mantle edges of *Corbicula* form a deep protective siphonal pocket and greaty narrow the pedal gape of the clam. The clam consequently is much less exposed (Fig. 3B). Moreover, the strong dentition of the heavy, rather inflated, ridged shell valves and the mantle sutures which "stitch" the lobes together to form a foramen for the adductor muscles (Kraemer, 1977), further protect the internal organs.

If spawning function is considered, a marked contrast between unionacean and sphaeriacean bivalves is observed. Distribution of the parasitic glochidial larvae has quite obviously become a function not only of the modified outer marsupial gills of female *Lampsilis* spp. and *Carunculina* spp., but also of the posterior mantle lobes. Mantle flaps and mantle ganglia of *Lampsilis* spp. function in the spawning process, as do the highly innervated, "thumb twiddling" caruncles and pulsing, membranous mantle edges of *Carunculina* spp.

On the other hand, reproduction in the monoecious sphaeriacean, *C. fluminea,* has involved elaborate innervation of the gonoduct lips via the cerebro-visceral connectives and development of a series of "follicular ganglia" associated with confluences of mature oogenic and differentiating spermatogenic follicles. These follicular ganglia, each innervated by nerves from nearby pedal or cerebral ganglia, are structures concomitant with the hermaphroditic process in *C. fluminea.* Thus, both the follicular ganglia of *C. fluminea* and the modified neuronal structures of *Lampsilis* and *Carunculina* spp. mantle edges clearly are associated with reproduction in the two very different kinds of animals.

Differences in the organization of the statocysts may be of more fundamental systematic significance. As organs implicated primarily in trophic activity of the organisms, conjoined statocysts of the relatively quick-moving *Carunculina* may signify a quite different environmental/adaptational history than that suggested by the widely separate, cislaterally innervated statocysts of the slower-moving *Lampsilis* spp. In *Lampsilis* spp., function of the statocysts also is implicated in the long "headstand" which accompanies the spawning process of flapping behavior.

These studies on the functional morphology of peripheral neural entities amplify an interesting peculiarity of molluscan neuronal organization *i.e.* the cell bodies of molluscan neurons are not confined to their ganglia. On the contrary, neuronal soma spread along many of a mussel or clam's nerves, and frequently aggregate in small clusters at the distal ends of even the smallest nerves. Prosser (1973: 648) comments ". . . nerve cell bodies are common in the peripheral nerves of many molluscs. . . ." Beyond such casual references, one searches the literature in vain for discussion of this phenomenon.

Functionally, what is the role of such peripheral aggregations of neuronal soma? Tauc (1966: 388) observed that ". . . if evolutionary rank may be assigned on the basis of dominance that the central nervous system exerts over peripheral events the Mollusca head the list of all invertebrates. . . ." That this view is not consistently adhered to is indicated by Prosser's comment (1973: 648) that ". . . in *Aplysia*, a gill disconnected from central ganglia contracts to local tactile stimulation and this response, mediated by peripheral plexus, can be habituated. . . ."[2]

As an alternative to the piecemeal approach to the study of mollusks, I have here tried to marshall evidence from neuroanatomical/histological and behavioral studies to argue that disparate and differently organized peripheral neural entities integrate the reproductive process in the monoecious sphaeriacean bivalve *Corbicula fluminea*, and in the essentially dioecious unionacean bivalves *Lampsilis* and *Carunculina* spp. Behaviorally, these animals are very different (Figs. 14, 15). Physiological data are needed to connect and verify the tentative conclusions presented here. Logistical difficulties associated with careful physiological study of such small clusters of unmyelinated neurons, however, render the latter less than appealing preparations for rigorous experimental exploitation. It also is humbling to realize that Bullock's comment in 1965 (:451) is still patently true: ". . . the gulf between our present level of physiological understanding and the explanation of behavior . . . is wider than the gulf between atomic physics and astronomy and is indeed the widest gap between disciplines in science. . . ."

[2]Such preparations have been much exploited by physiologists. For example, Lukowiak & Sahley (1981) report that they have demonstrated "associative learning" in a reduced-siphon-mantle-gill-abdominal ganglion preparation of *Aplysia*.

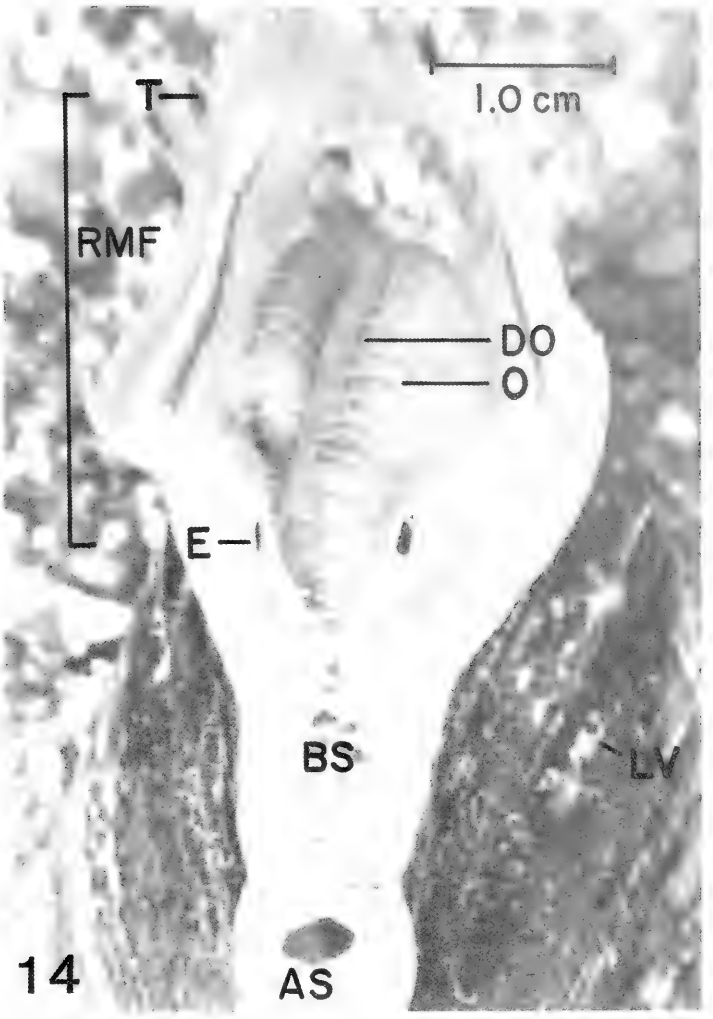


FIG. 14. Photograph from posterodorsal aspect of *Lampsilis ventricosa*, living mature gravid female, during flapping behavior. Note that posteroventral surfaces of both charged marsupial gills are pushed out of the mantle cavity and between the mantle flaps (E, T). The dark line in the distal edge (DO) of each ovisac (O) indicates the location of the "pores" seen by Kraemer (1970) through which glochidia appear to be discharged.

As a final comment, one may observe that the soft bodies of mollusks provide the animals with a neuroanatomical context within which the plasticity and variability of the molluscan nervous system may be exploited. This is demonstrably true of the developmental twisting of gastropod nervous systems and of the neurophysiological virtuosity displayed by the large, sentient surface of cephalopods. It also is observable among the bivalves, the *acéphales,* which eschewed an anterior "brain" and developed large, fused ganglia at their posterior ends along with an extensive system of peripheral nerves. It seems reasonable to expand investigations into the organization and function of various molluscan nervous systems as a means of interpreting the phylogenetic history of the higher taxa. Such studies also offer, *sui generis*, the opportunity for their pursuers to understand adaptational capacity and to predict direction of response to environmental change among the higher taxa of mollusks.

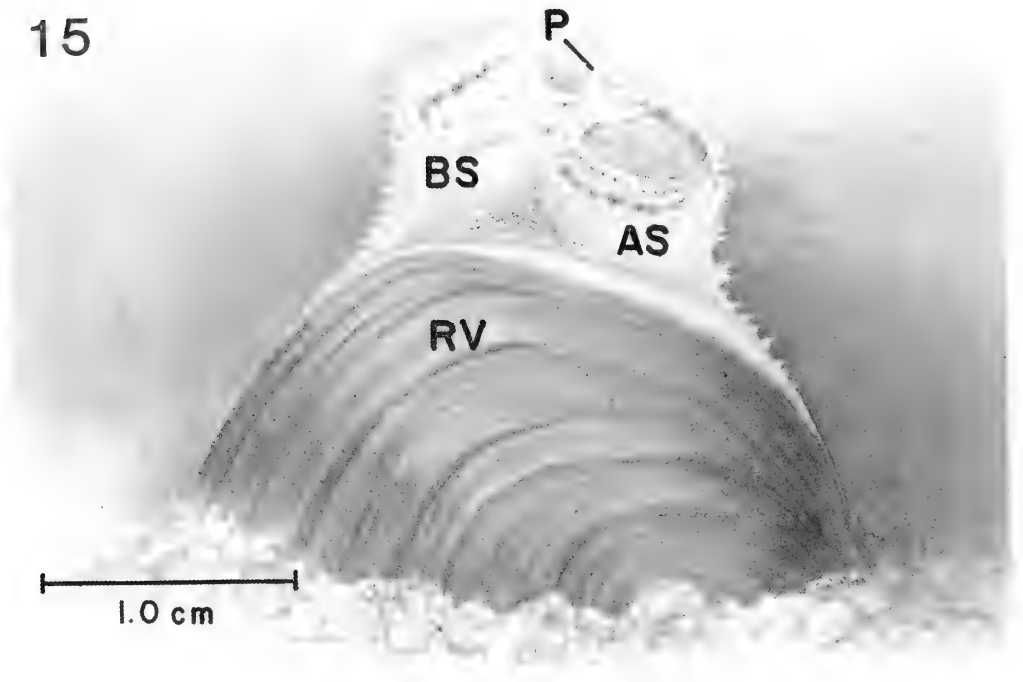


FIG. 15. Drawing of living *Corbicula fluminea* showing characteristic appearance of the animal's muscular siphons which protrude between the shell valves as tubes from the siphonal pocket within.

ACKNOWLEDGEMENTS

I thank Professors H. van der Schalie, D. Dundee, E. H. Schmitz and E. Russert Kraemer for their critical review of the manuscript.

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I am also happy to thank Julia Renner whose competence and good humour were more than a match for a number of versions of the typed manuscript of this paper.

LITERATURE CITED

BULLOCK, T. H., 1965, Physiological bases of behavior. *In* MOORE, J. A., ed., *Ideas in Modern Biology. Proceedings XVI International Congress of Zoology*, Natural History Press, New York, 6: 451–482.

BULLOCK, T. H. & HORRIDGE, G. A., 1965, *Structure and Function in the Nervous Systems of Invertebrates*. II. Freeman, San Francisco, 1611 p.

BURCH, J. B., 1973, Freshwater unionacean clams (Mollusca: Pelecypoda) of North America. *Biota of Freshwater Ecosystems Identification Manual No. 11,* Environmental Protection Agency Document, Washington, D.C., 176 p.

CLARKE, A. H., 1973, The freshwater molluscs of the Canadian interior basin. *Malacologia*, 13: 1–509.

DAVIS, G. M. & FULLER, S. L. H., 1981, Genetic relationships among recent Unionacea (Bivalvia) of North America. *Malacologia*, 20: 217–253.

DUVERNOY, M., 1852, Mémoires dur le système nerveux des mollusques acéphales. *Mémoires de l'Académie des Sciences de l'Institut de France, Paris,* 24: 1–210.

FRETTER, V. & GRAHAM, A., 1964, Reproduction, *In* WILBUR, K. M. & YONGE, C. M., eds., *Physiology of Mollusca*. Academic Press, New York, 1: 127–164.

HEARD, W. H. & GUCKERT, R. H., 1970, A re-evaluation of the recent Unionacea (Pelecypoda) of North America. *Malacologia,* 10: 333–356.

HUNTER, W. R., 1964. Physiological aspects of the ecology of nonmarine mollusks. *In* WILBUR,

K. M. & YONGE, C. M., eds., *Physiology of Mollusca,* Academic Press, New York, 83: 126.

JOHNSON, R. I., 1970, The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the Southern Atlantic Slope region. *Bulletin of the Museum of Comparative Zoology at Harvard University,* 140: 263–449.

KRAEMER, L. R., 1970, The mantle flap in three species of *Lampsilis* (Pelecypoda: Unionidae). *Malacologia*, 10: 225–282.

KRAEMER, L. R., 1974, Flapping behavior and its species modifications in a group of freshwater mussels (Bivalvia: Unionidae: Lampsilinae). *First International Congress of Systematics and Evolutionary Biology, Abstract,* University of Colorado, Boulder, Colorado, U.S.A., no pagination.

KRAEMER, L. R., 1977, Aspects of the functional morphology of the mantle/shell and mantle/gill complex of *Corbicula* (Bivalvia: Sphaeriacea: Corbiculidae). *Bulletin of the American Malacological Union,* 1977: 25–31.

KRAEMER, L. R., 1978a, Discovery of two kinds of statocysts in freshwater bivalved mollusks: some behavioral implications. *Bulletin of the American Malacological Union,* 1978: 24-28.

KRAEMER, L. R., 1978b, *Corbicula fluminea*: (Bivalvia: Sphaeriacea): The functional morphology of its hermaphroditism. *Bulletin of the American Malacological Union,* 1978: 40–49.

KRAEMER, L. R., 1979, *Corbicula* (Bivalvia: Sphaeriacea) vs. indigenous mussels (Bivalvia: Unionacea) in U.S. rivers: a hard case for interspecific competition? *American Zoologist,* 19: 1085–1096.

KRAEMER, L. R. & LOTT, S., 1977, Microscopic anatomy of the visceral mass of *Corbicula* (Bivalvia: Sphaeriacea). *Bulletin of the American Malacological Union,* 197: 48–55.

LUKOWIAK, K. & SAHLEY, C., 1981, The in vitro classical conditioning of the gill withdrawal reflex of *Aplysia californica. Science*, 212: 1516–1518.

ORTMANN, A. R., 1911, A monograph on the Naiades of Pennsylvania. Pts. 1 and 2. *Memoirs of the Carnegie Museum,* 4: 279–374.

PROSSER, C. L., 1973, *Comparative Animal Physiology,* ed. 3. Saunders, Philadelphia, 966 p.

RAWITZ, B., 1887, Das Zentralnerven system der Acephalen. *Jenaische Zeitschrift für Naturwissenshaft,* 20: 285–457.

SCHALIE, H. VAN DER, 1952, An old problem in naiad nomenclature. *Nautilus*, 65: 93–99.

SCHALIE, H. VAN DER, 1970, Hermaphroditism among American freshwater mussels. *Malacologia*, 10: 93–109.

SIMPSON, C. T., 1914, *A Descriptive Catalogue of the Naiades or Pearly Freshwater Mussels, Part I, Unionidae*. Ann Arbor Press, Ann Arbor, Michigan.

TAUC, L., 1966, Physiology of the nervous system. In WILBUR, K. M. & YONGE, C. M., eds., *Physiology of Mollusca*. Academic Press, New York, 2: 387–454.

WALKER, B., 1917, The method of evolution in the Unionidae. *Occasional Papers of the Museum of Zoology, University of Michigan*, No. 45: 1–10.

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THE PARAGASTROPODA: A PROPOSAL FOR A NEW CLASS OF PALEOZOIC MOLLUSCA

Robert M. Linsley[1] & William M. Kier[2]

ABSTRACT

A functional analysis of the shells of the hyperstrophic and other apparently left-handed "gastropods" of the Paleozoic suggests that these are not the shells of torted mollusks. They should not, therefore, be considered gastropods, and as they do not fall within even a broad concept of Monoplacophora we suggest that they be considered members of a new class of mollusks. This group includes the following taxa which have previously been placed in the class Gastropoda: Onychochilidae, Macluritidae, Pelagiellidae, Clisospiridae and possibly the Euomphalacea. The following new taxa are proposed: class—Paragastropoda: orders—Orthostrophina, Hyperstrophina; family—Aldanellidae.

INTRODUCTION

Among living univalve mollusks, only members of the class Gastropoda have anisostrophically coiled shells. Thus it has been presumed that all anisostrophic shells in the geologic record must, perforce, be gastropods. In 1952, on considering the shells of the Lower Cambrian genus *Pelagiella*, that astute observer of Paleozoic Gastropoda, J. Brookes Knight (Knight, 1952: 43) wrote: "I . . . [doubt] . . . that they are gastropods." However, he was not able to verbalize just what was "ungastropod" about these enigmatic shells. Recent attempts (Linsley, 1977) to reconstruct the Lower Paleozoic genus *Onychochilus* as a gastropod resulted in a rather strange looking beast (Fig. 1) and provided insight into how *Onychochilus* and *Pelagiella* differ in their appearance from typical gastropods that can be studied today.

One of the most obvious ways that *Pelagiella* and *Onychochilus* differ from modern gastropods is in the shape of the aperture. In both genera the aperture is elongated but the long axis of the aperture is oriented at approximately right angles to elongated apertures of modern gastropods. In making the reconstruction of *Onychochilus,* Linsley ended up with an organism whose shell was oriented in such a way that the long axis of the aperture was almost at right angles to the long axis of the foot. Yet according to a more recent study (McNair *et al.,* 1981) almost all modern gastropods orient their shell so that the long axis of the aperture is subparallel to the long axis of the foot. Obviously with any given shell the head of the mollusk could be interpreted as being at either end of the aperture. In the case of *Onychochilus* if the head was at the basal end of the aperture, the organism would be interpreted as a left-handed, orthostrophic gastropod. If, however, the head of *Onychochilus* was located at the spire end of the aperture, the organism would be right-handed, hyperstrophic but untorted (Fig. 2).

It is the purpose of this paper to demonstrate that this interpretation of the shells of many Paleozoic molluscs is sound and in fact makes comprehensible many previously unexplained aspects of these shells.

TORSION IN THE GASTROPODA

Gastropods, by definition, are mollusks which have undergone torsion or are descended from torted ancestors. Torsion is a rather unusual process which results in rotation of the shell and its contained viscera 90° to 180° relative to the foot and head of the gastropod. Mechanically, torsion is a relatively simple process in that only one of a pair (primitively) of velar retractor muscles begins to function and this unbalanced pull results in rotation of the shell (Knight, 1952; Eales, 1950; Crofts, 1937; Smith, 1935). The functional significance of this process has been

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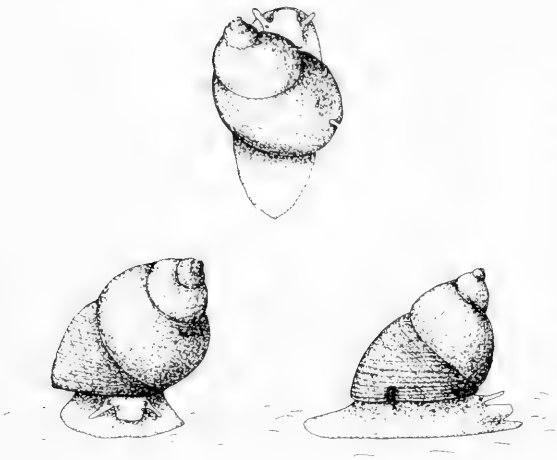

FIG. 1. Reconstruction of *Onychochilus* as a gastropod (Linsley, 1977).

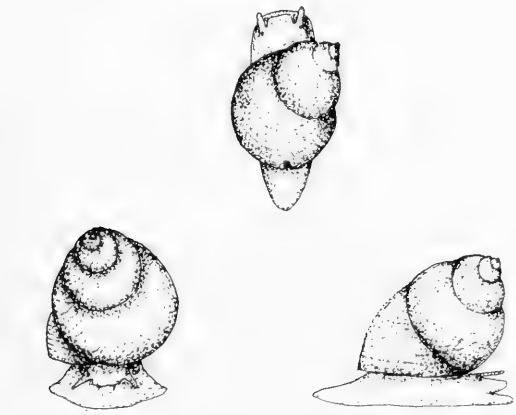

FIG. 2. Reconstruction of *Onychochilus* as a paragastropod.

the subject of much discussion (Ghiselin, 1966; Batten, Rollins & Gould, 1966; Runnegar & Pojeta, 1974; Thompson, 1967; Linsley, 1978a) and there seem to accrue advantages for both the larva and the adult. The immediate advantages are the result of the mantle cavity being brought over the head. This allows the larva to retract the velum into the mantle cavity (Garstang, 1928) and the adult to more effectively clamp the shell over the head region. Subsequent advantages for the adult result from the fact that once the mantle cavity is situated anteriorly it is possible to envelop a larger, more complex head and improved circulation patterns (Linsley, 1978a).

It is frequently suggested (e.g. Eales, 1950) that torsion is the cause of asymmetry of gastropods. We believe that this is an unwarranted assumption. The process of torsion and anisostrophic coiling are completely separate events, with no causal connection between them. For example, it has been argued (Knight, 1952; Horný, 1963; Yochelson, 1967; Rollins & Batten, 1968; Peel, 1974; Linsley, 1978a) that the bellerophonts are symmetrical, yet torted gastropods. Further, the monoplacophoran *Cyrtonella* has marked asymmetry. We suggest in this paper that there are numerous asymmetrical untorted mollusks. We will attempt to show that there are problems that attend isostrophic shells of more than one volution and that anisostrophism is a possible solution to this problem in both torted and untorted molluscs.

The great majority of modern gastropods are right-handed (dextral) although left-handed (sinistral) shells are fairly common. Classically, if a shell is held with the spire up and the aperture facing the observer, then the aperture will be on the right in a dextral shell and on the left in a sinistral shell. In right-handed shells the right gill is frequently lost and the anus migrates in the direction of the lost gill or towards the upper suture. Other organs, which are primitively paired, also tend to lose one of the pair during evolution. Left-handed gastropods are mirror images of right-handed gastropods, both internally and externally. Thus in a left-handed gastropod it would be the left gill that is lost. However, there also exist species that have the soft anatomy of a right-handed gastropod in an apparently left-handed shell. In these forms the anus appears to migrate away from the apex of the shell rather than towards the spire. Because of this we tend to illustrate these hyperstrophic shells with their aperture on the right as we would a right-handed shell. This has the consequence of placing the spire in a "down" position, and hence the descriptive term, "depressed spire," is used in reference to hyperstrophic shells.

It might make understanding sinistrality and dextrality of Gastropoda easier if we defined these terms relative to the process of torsion itself. Dextral gastropods are defined as those whose left velar retractor muscle aborted (or delayed development) thus causing the right retractor muscle to produce torsion so that the shell turned in a counter-clockwise manner relative to the foot as viewed from above. In sinistral gastropods it is the left retractor that

produces torsion and the shell swings in a clockwise fashion. This definition would allow us to talk about right and left-handed limpets and bellerophonts although we cannot think why anyone should. Perhaps asymmetry of the deep retractor muscles of the bellerophonts would allow us to refer to them in this way.

Recognition of hyperstrophic, orthostrophic, dextral, sinistral, torted and untorted conditions is fairly straightforward with an organism with soft parts. Then it is possible to compare the positions of internal anatomical features relative to shell geometry. However, recognition of these conditions in the fossil record is a different story. Dextrality and sinistrality (and hence orthostrophy and hyperstrophy) can be recognized by the "rule of opercula" (Cox in Knight *et al.,* 1960: I125). In dextral gastropods the operculum, if spiral, always grows in a counterclockwise direction when viewing the exposed side of the operculum. Thus the accreting margin is placed against the parietal wall of the aperture when the animal is retracted. Conversely in sinistral forms, growth of the operculum is always clockwise.

Recognition of torsion in fossils is an even more difficult problem. Because of the "bellerophont problem" all of our attention has focused on the recognition of torsion in isostrophic forms. This problem is treated in existing literature (Rollins & Batten, 1968; Linsley, 1978a) and since it is not directly pertinent to the present problem, we will not review it here. The recognition of torsion in anisostrophic shells has never been considered because of the unwarranted assumption that anisostrophy is a necessary consequence of torsion. Thus it has always been assumed that with the exception of *Cyrtonella*, all anisostrophic "gastropod" shells were necessarily torted. Obviously, some mollusk shells, such as those of turriliticone cephalopods are anisostrophic without being torted. Almost equally obviously, not all coiled shells are those of mollusks, some foraminifera and serpulid worms being examples.

THE RULE OF APERTURAL ELONGATION AND ITS APPLICATION TO ORTHOSTROPHY AND HYPERSTROPHY

Linsley (1977) has demonstrated that gastropods support their shells over their backs so that the shell is balanced. McNair *et al.* (1981) have demonstrated that in gastropods with elongate apertures (major axis greater than 20% of minor axis), the shell is oriented with the major axis subparallel to the long axis of the foot or the antero-posterior axis of the organism. If these two generalizations are valid, they provide a possible means of recognizing torsion in a fossil, anisostrophic mollusk.

In right-handed, orthostrophic torted mollusks (the majority of modern gastropods) the spire of the shell projects to the right side of the animal and is swung towards the posterior by regulatory detorsion (Fig. 3). If a gill is eliminated, it is always the right gill and the anus migrates to the spire side of the aperture or the functional posterior portion of the aperture. Reasoning by homology, an untorted, right-handed, orthostrophic mollusk would have the spire protruding to the left side of its body and would balance the shell by swinging the spire backwards (Fig. 4), though in this instance it would be called "regulatory torsion" rather than "regulatory detorsion." If a gill were lost, it would be the left gill (which would become the right gill if torsion were to occur) and the anus would migrate to the spire side of the aperture or the functional posterior. The concept of "orthostrophy" in untorted molluscs is thus defined by homology with torted orthostrophs. Functionally, water currents will enter the base of the aperture and exit at or near the suture in orthostrophic mollusks.

In torted hyperstrophic mollusks the spire projects to the left side of the animal. Regulatory detorsion presumably swings the spire over the head of the animal (Fig. 3), but it is the right gill that is lost and the anus migrates abapically to the "base" of the aperture, which is now functionally posterior. By homology, in untorted hyperstrophic mollusks the spire projects to the right side of the organism and is swung forward over the head by regulatory torsion (Fig. 4). Elimination of the left gill (the torsional right gill) allows the anus to migrate abapically which is again functionally posterior. Again, "hyperstrophy" in untorted mollusks is defined by homology with their torted counterparts. Functionally, water currents in hyperstrophs will enter at or near the sutural portion of the aperture and exit at the base of the aperture in both torted and untorted forms. As a result of the shell balancing process, the long axis of the aperture of torted anisostrophic mollusks will be at right angles to the long axis of their counterparts in un-

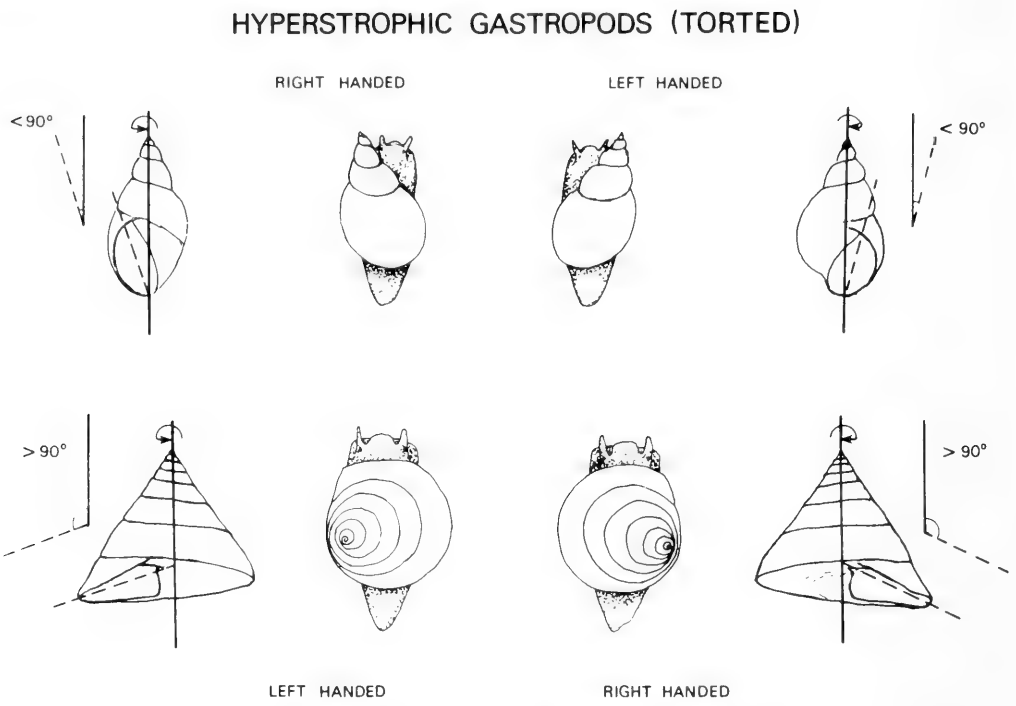

FIG. 3. The shell balancing process in gastropods places the spire of the shell to the posterior in orthostrophic forms and to the anterior in hyperstrophic forms. In all cases the gill and inhalant currents will be anteriorly positioned while the anus and exhalant currents will be posteriorly positioned. Since the aperture elongates sub-parallel to the long axis of the foot the two lines formed by the axis of coiling and the long axis of the aperture will form an acute angle in hyperstrophic forms and an obtuse angle in orthostrophic forms.

torted anisostrophic mollusks. Thus hyperstrophic untorted shells will tend to resemble orthostrophic torted shells in terms of apertural elongation and orthostrophic untorted shells will have apertures that elongate like those of hyperstrophic torted shells (Figs. 3 and 4).

The preceding analysis is made on the assumption that there will be direct homology between torted and untorted forms. The abundance of living prosobranch gastropods support the model of right and left-handed orthostrophic torted forms. For hyperstrophic forms our sources of confirmation are restricted indeed. Among the living hyperstrophic forms are *Lanistes* (right-handed) and *Carinifex (Carinifex) newberryi* Lea (left-handed). We have only had the opportunity to observe a single specimen of *Lanistes* in motion. When that *Lanistes* moved ahead in a straight line, the axis of coiling of its shell was only slightly inclined to the substrate and roughly at right angles to the long axis of its foot. One other "living" hyperstrophic form is a single live-collected shell of the prosobranch *Heliacus* described by Robertson & Merrill (1963). *Heliacus* normally has a hyperstrophic larva which reverts to orthostrophy in the adult. The one individual reported by them continued larval hyperstrophy into adulthood. Robertson & Merrill (1963) inferred that the anus of this form was indeed at the base of the shell and that inhalant currents entered the aperture near the suture. Thus the spire of this animal projected in front of the head, consistent with our model. It should be noted that the form of this aberrant *Heliacus* is that of a shell dragger, snails whose center of gravity is so far displaced from the aperture that they cannot support their shell over their back. Shell draggers are particularly oblivious to the relative position of their shell to their

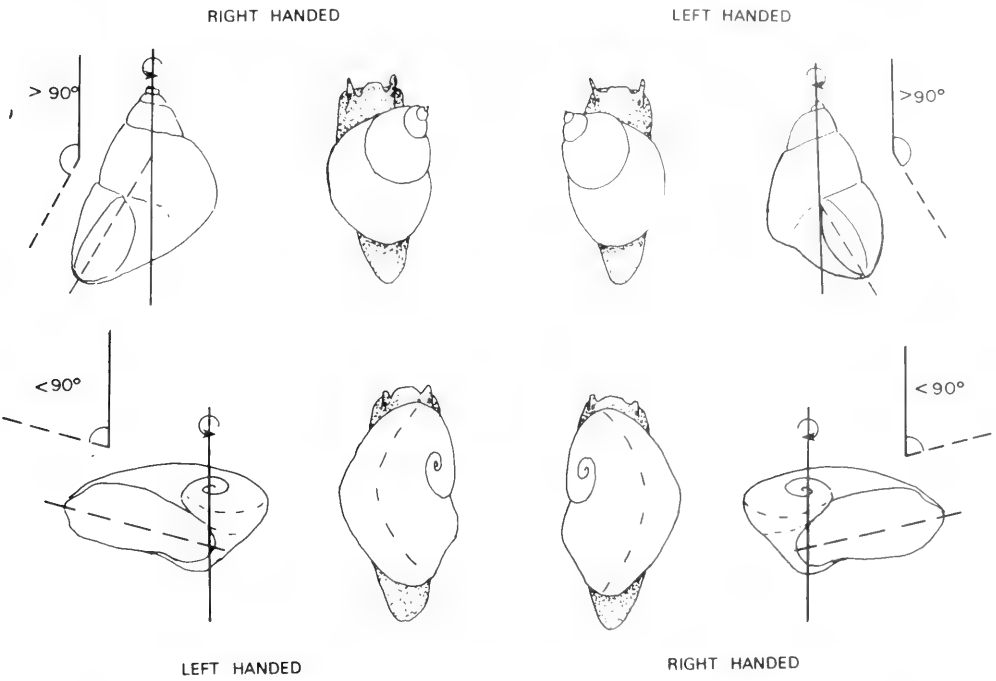

FIG. 4. The shell balancing process in paragastropods places the spire of the shell to the posterior in orthostrophic forms and to the anterior in hyperstrophic forms. The gill and inhalant currents are presumed to be anteriorly placed in all instances while the anus and exhalant currents are situated posteriorly. Again the long axis of the aperture is subparallel to the long axis of the foot with the result that an acute angle will be found between the long axis of the aperture and the axis of coiling in orthostrophic forms, but it will be an obtuse angle in hyperstrophic forms.

body, seemingly as comfortable with the shell being pushed in front of them or stuck straight out to the side as they are holding it in the normal straight back position (Linsley, 1977). And since *Heliacus* is near sessile because of its mucous thread (Robertson & Merrill, 1963) it was apparently not too distressed by the abnormality and managed to survive until adulthood. Thus it can be seen that a few examples from the living world are consistent with the rule of apertural elongation, but lack of variety does not allow the living world to be very supportive.

The rule of apertural elongation cannot be applied indiscriminately. For example, the rule cannot be invoked for forms with essentially round apertures (when the long axis is less than 1.2 times the minor axis). In addition, the rule cannot be applied in cases where balancing is accomplished by regulatory detorsion (or regulatory torsion in untorted forms) that approaches 90°. As the axis approaches 90°, torted and untorted forms tend to converge and resemble each other. Finally, the rule cannot be invoked for sessile forms, forms with radial apertures, or any forms that do not balance their shell during locomotion.

THE PALEONTOLOGICAL RECORD

There are many fossil shells that seem to possess the shapes of untorted mollusks. These groups that we can identify with certainty include the Pelagiellidae, and the Onychochilidae. There are also other groups that we believe are untorted, but since they either have circular apertures or radial apertures we cannot be certain. These include the

enigmatic lower Cambrian genus *Aldanella*, the Macluritacea and the Euomphalacea.

Of all of these above-mentioned groups, the Onychochilidae, including *Onychochilus* Lindström and its relatives *Matherella* Walcott, *Matherellina* Kobayashi, *Laeogyra* Perner, *Sinistracirsa* Cossmann, *Kobayashiella* Endo, *Pervertina* Horný, *Invertospira* Horný, *Helicotis* Koken, and *Hyperstrophema* Horný, constitute the largest and best-known group because of the relatively well-preserved shells of various of these genera.

The onychochilids possess a number of features that seem very unusual for gastropods, but that are consistent with an interpretation of them as untorted organisms. First, all appear to be left-handed, and have been long interpreted as being hyperstrophic, a position with which we concur. However, the evidence for this is circumstantial, for no opercula have ever been found associated with any of these genera. The interpretation of this group as hyperstrophic rests on their presumed relationship with the Macluritacea which are known to be hyperstrophic because of evidence from opercula associated with their shells. The basis of the relationship of the macluritids and onychochilids rests on the fact that: 1) these are the only apparent "sinistral" forms in a Lower Paleozoic world comprised otherwise only of right-handed forms; 2) apertural shapes are quite similar (and both strangely distinct from known gastropods); 3) both have a distinctive umbilicus which is unusual when compared to most gastropods; and 4) both groups have an angulation in their apertural form which has been interpreted (Knight, 1952) as marking the position of the anus. We agree that these similarities are sufficient to establish the relationship between these two groups and thus warrants the interpretation of the onychochilids as being hyperstrophic.

In addition to being hyperstrophic, all onychochilids have prosoclinal growth lines inclined at an unusually steep angle. From this we infer that during locomotion the axis of coiling was steeply inclined to the substrate (Fig. 2). Within living gasropods the low-spired trochids have comparably steep prosocline apertures. In onychochilids we find this feature in varied geometries from the low-spired *Kobayashiella* (where it is more or less expected) to the very high-spired *Sinistracirsa* and *Matherella* where it is most unlike modern gastropods. We feel that this high angle of inclination is a necessary adaptation to hyperstrophy. Since the inhalant currents come into the aperture anteriorly and the spire is projected anteriorly, the spire of the shell has to be lifted high off the substrate to accommodate these currents.

The strangely-shaped umbilicus, unusually deep and wide when compared to gastropods of a similar geometry, can also be interpreted as an accommodation to the inhalant current. In modern gastropods the base of the shell is functionally the anterior in the majority of mobile forms, hence an open umbilicus is a source of turbulence and is rarely found in modern mobile snails. The only living gastropods with wide, open umbilici are relatively immobile ones or those who hold their coiling axis with a high inclination so that the umbilicus is functionally in a ventral position and is hidden against the upper surface of the cephalopedal mass. The base of the shell, including the umbilical area, is occasionally sculpted to accommodate a large calcareous operculum which essentially locks into place. In the onychochilids the umbilicus is functionally ventral in the living animal and occupies a position in front of the aperture. Thus a broad open umbilicus would open up this area of the shell to the anteriorly directed inhalant current, which would presumably enter near the suture.

The final unusual feature of the onychochilids which is made explicable by the interpretation of them as untorted hyperstrophs is the strangely-shaped aperture. In many onychochilids it is banana-shaped with the inner lip (which forms the umbilicus) bending abaxially to constrict the aperture on the columellar side. The aperture is generally extended or angulated at its posterior end which is interpreted as marking the position of the anus. The holotype of *Onychochilus physa* Cossmann, which is one of the best preserved specimens of the family, shows a re-entrant at the posterior portion of the lip. If this re-entrant is not an artifact of preservation, then this was strictly a feature of the adult shell, for it generates no selenizone. We do not think that it is an artifact, for there is a raised and reinforcing deposit of shell material around the re-entrant. We feel quite certain that this is an anal re-entrant which in the reconstructed living organism would be positioned in the posterior-most position, a convenient place to have an anus.

The second group that we feel certain is untorted is the Cambrian family Pelagiellidae. We interpret these shells as belonging to

right-handed orthostrophic untorted mollusks (Fig. 4, orthostrophic). In the type-species, *Pelagiella atlantoides* Matthews, the aperture is elongated at almost right angles to the coiling axis of these shells and as Knight noted (1952: 43), "I . . . [doubt] . . . that they are gastropods." Many pelagiellids have two sinuses in the aperture. One of these is typically positioned at or just above the shell periphery, as in *Cambretina mareki* Horný. In our reconstruction of this organism this sinus is in a posterior position and is interpreted as an anal re-entrant which sometimes generates a selenizone. The other re-entrant is typically located on the abapical portion of the shell and is here interpreted as the inhalant re-entrant, since it would be positioned anteriorly in our reconstruction.

We have not seen any muscle scars associated with these shells, but would expect them to be multiple and are likely to be asymmetrical due to the anisostrophism.

Another genus which deserves comment along with *Pelagiella* is the Lower to Middle Cambrian genus *Aldanella*. It is quite possible that these shells are not molluscan at all (Yochelson, 1978), but if they are, we suspect that they are shells of an untorted mollusk. Unfortunately, this can be no more than a suspicion for the apertures are not preserved, nor are growth lines from which apertural form could be deduced. At the moment of writing it is not even known if *Aldanella* has a radial or tangential aperture. If it is a radial aperture, then we would certainly agree with Yochelson that these are not molluscan. If they possess a tangential aperture, we feel it would greatly increase the probability of a molluscan affinity, for a tangential aperture implies a dorsal shell. If *Aldanella* should prove to have molluscan affinities, we would suspect an untorted condition but cannot demonstrate it at this moment because *Aldanella* has a circular whorl cross-section and we cannot invoke the rule of apertural elongation to demonstrate torsion or non-torsion.

The next group of possibly untorted mollusks is the Macluritidae, including the genera *Maclurites* Lesueur, *Palliseria* Wilson, *Scaevogyra* Whitfield, *Macluritella* Kirk, *Antispira* Perner, *Teiichispira* Yochelson & Jones, and *Versispira* Perner. The genus *Lecanospira* Ulrich & Bridge may be a macluritid, but we are more inclined to believe that they are euomphalids. All members of this group have radial apertures and are presumed to lie with one side of their shell on the substrate (Linsley, 1978b). Thus the rule of apertural elongation cannot be invoked for this group. However, as mentioned above, other characters such as hyperstrophy, unusual umbilicus and general aperture shape, allow the establishment of affinities with the Onychochilidae and thus attest to the untorted nature of this group.

The Euomphalacea present a more difficult problem. There are strong similarities between the Macluritidae and the Euomphalacea, in that both groups have essentially a discoidal whorl form and a radial aperture, frequently with the suggestion of a sinus (presumably exhalant) on the "uppermost" surface of the whorl. However, it is possible that these are the result of convergence with two groups independently adapting to the sedentary suspension-feeding niche. Since the euomphalaceans have circular whorl profiles *and* radial apertures, it is impossible to invoke the rule of apertural elongation to infer the condition of torsion. However, we suspect that the similarities between these two groups are not those of convergence but of common descent and would suggest that they may indeed be untorted.

The final group to be considered as a possible candidate for untorted, anisostrophic mollusks is the Clisospiridae, including the genera *Clisospira* Billings, *Mimospira* Koken, *Ferrogyra* Horný, *Conoclisa* Horný, *Trochoclisa* Horný, *Antigyra* Horný, *Antizyga* Horný, *Atracura* Horný, *Bodospira* Wängberg-Eriksson, *Angulospira* Wängberg-Eriksson, *Tapinogyra* Wängberg-Eriksson, and *Undospira* Wängberg-Eriksson. This is a poorly known group, but Upper Ordovician specimens recently described from Sweden (Wängberg-Eriksson, 1979) strongly suggest that they are closely related to the Onychochilidae and should be considered untorted. They are all hyperstrophic and have the same elongated aperture and broad umbilicus as have the Onychochilidae. Horný (1964) noted the similarities between the Clisopiridae and the Onychochilidae and recommended that both be subsumed under the Onychochilidae.

PHYLOGENY

We suspect that the Paragastropoda are polyphyletic and that the orthostrophic pelagiellids are not related to the hyperstrophic onychochilids. Thus we look upon the class

Paragastropoda as being a grade of organization rather than a clade.

Anisostrophy is regarded as a solution to the problems attendant to isostrophic coiling rather than torsion. If a mollusk with a dorsally situated shell develops an isostrophically coiled shell with the spire placed over the head of the organism, then this coiled mass and the body stalk of the animal will effectively block inhalant currents from the anterior position (Linsley, 1978a). As a result, cyclomyan monoplacophorans of more than one volution all show the development of angulations of the lateral apertural margins to accommodate laterally placed inhalant currents. Torsion was one solution to this problem for it eventually allowed the inhalant currents to move anteriorly into the now forward-placed mantle cavity (Linsley, 1978a). But even after torsion had produced the bellerophontids, however, anisostrophy eventually produced an even better solution by placing the left gill in a more anterior and more favorable position. The Paragastropoda provide a second solution to the problem of the laterally displaced inhalant currents. The development of anisostrophy causes a repositioning of the shell through the shell balancing process. Either orthostrophy or hyperstrophy serves to place the pretorsional right gill in an anterior position. The fact that both *Pelagiella* and *Onychochilus* have elongated apertures strongly suggests that both have lost the left gill.

It is obvious that once this adaptation had occurred, these animals must have been an evolutionary dead end. They could not serve as ancestors to the gastropods because torsion would serve to place the anus in front of the gill and necessitate a complete reordering of water current through the mantle cavity.

The Pelagiellidae were Lower and Middle Cambrian experiments which underwent a limited radiation, but, as inferred from their tangential aperture, never advanced beyond a mobile browsing form. In contrast, the Onychochilidae, which first appears in the Upper Cambrian, and are inferred from their shell to be mobile browsers, not only persisted in that form but had radiations into two other niches as well. In one major alteration of the basic body plan, the Macluritidae rested their shell on the right side and took up a sedentary mode of life as suspension feeders (Linsley, 1978b). This proved a very successful adaptation and the family is very abundantly represented in Ordovician rocks.

If this group was ancestral to the Euomphalacea as suggested in the "Treatise" (Knight *et al.,* 1960), then this filter feeding adaptation persisted throughout the Paleozoic and was represented in almost every quiet water habitat.

The second major adaptation is assumed by the Clisospiridae and it is one that we do not fully understand. The group includes both low-spired forms like *Clisospira* and *Ferrogyra* as well as high-spired genera like *Atracura, Mimospira,* and *Antizyga.* The low-spired group has a frilled extension around the base and is obviously adapted to holding the shell with the coiling axis highly inclined to the substrate and the base pressed against the substrate. It should be noted that while the macluritids rest their right side on the substrate so that the "spire" is down, the Clisospirids rest their left side against the substrate so that their "spire" is up. As such they are reminiscent of the Pseudophoridae and may have made comparable adaptations (Linsley, Yochelson & Rohr, 1978). The high-spired group is quite puzzling, however, for living gastropods of comparable spire height are all shell draggers, allowing their shell to rest on the substrate behind the cephalopodal mass during locomotion. Yet the highly prosocline aperture and excavated base of the high-spired clisospirids suggests that the shells of these animals were positioned directly over the animal's back so that the axis of coiling is highly inclined relative to the substrate. This would present the organism with a very highly-placed center of gravity which would likely preclude much movement (Linsley, 1978b). It would also be a disadvantageous shell form in an area of any appreciable currents. Possibly, like the Macluritidae, the Clisospiridae were suspension feeders.

TAXONOMIC IMPLICATIONS

The major conclusion of this study is that some groups of Paleozoic shells belong to animals that have not undergone torsion. These groups include the orthostrophic pelagiellids, and the hyperstrophic macluritids, onychochilids, and clisospirids. The euomphalids are included in the discussion because of their resemblance to the macluritids. However, this resemblance may be one of convergence of two very disparate groups rather than phyletic affinity.

The next problem is to determine the sig-

nificance of a group of asymmetrical untorted molluscs. We could: (1) re-define the concept of Gastropoda to accommodate these organisms; (2) we could also re-define the class Monoplacophora for a similar purpose; or (3) we could erect a new class for this strange group.

While there is no single approach which will please every taxonomist, it is our conclusion that these animals should be accommodated in a new class. This conclusion is dictated by the two-fold consideration of our concept of the mechanism of evolution and the significance of the concept of "class." We are quite convinced that evolution proceeded in a mosaic fashion as suggested by Valentine (1979). This model suggests that it was relatively easier to achieve a class rank distinction in the early Paleozoic and progressively more difficult to do so.

One determinant of class rank would be the successful invasion of a new ecological sphere (Valentine, 1979). In the Lower Paleozoic this would be relatively easy since the major habitats were either unoccupied or occupied by organisms with relatively modest adaptive capabilities that offered relatively low-level competition. Thus new classes appear abundantly in the Lower Paleozoic, but with time and perfected adaptations the introduction of a new class becomes progressively more difficult and consequently less frequent.

We envision the early evolution of the Mollusca (the Lower and Middle Cambrian) as experiments taking place in an essentially predator-free sea. As such, the shell served primarily as protection from environmental factors rather than an anti-predation device. In this environment we see two major adaptations (classes) having taken place: the epifaunal molluscs (Monoplacophora) and the infaunal molluscs which have brought the shell down around their gills to protect them from fouling during burrowing (Rostroconcha). In addition there are a number of forms (helcionellids and yochelcionellids) that make no sense as either rostroconchs or monoplacophorans and probably should be accorded class rank. But until we understand the adaptive significance of their shell form and their mode of life, this would seem unwise.

The Upper Cambrian is marked by the advent of predation as produced by the introduction of cephalopods and possibly fish and even some gastropods. The Monoplacophora reflected this circumstance by surviving either as limpet-shaped forms with presumed low mobility or multiple-whorled, isostrophically coiled forms with a tangential aperture (such as *Cyrtolites*). The latter forms would presumably have greater mobility (Linsley, 1978b). Both of these forms would be restricted to rocky substrates where they could gain protection by clamping. In contrast the Paragastropoda (Onychochilida) and Gastropoda affected deep withdrawal into the shell and were not dependent on clamping against a firm substrate for protection. They were thus able to move out onto sediments. Eventually both presumably evolved opercula to augment the protection afforded by deep withdrawal into their shells. We presume that the gastropods with an anteriorly located aperture and orthostrophic or isostrophic shell were eventually to prove better adapted to this mode of life of browsing on soft sediments. The Paragastropoda, with their posteriorly located apertures and hyperstrophic shells survived only by moving into still another niche, that of essentially sessile, epifaunal suspension feeders. One group, the clisospirids came to rest on their left side while the macluritids came to rest on their right side. The euomphalids resemble the macluritids in their shell form and presumed life-mode and if they are descended from the macluritids then the major successful adaptation of the Paragastropoda was as epifaunal suspension feeders. It is because the Paragastropoda occupy a very different niche from the ancestral Monoplacophora that we feel that they deserve recognition as a new class of the phylum Mollusca rather than aberrant Gastropoda or Monoplacophora.

SYSTEMATIC PALEONTOLOGY

Phylum MOLLUSCA Cuvier, 1797

Class PARAGASTROPODA Linsley & Kier, new class

Diagnosis—Anisostrophically coiled, untorted mollusks. Shells either hyperstrophic or orthostrophic. Members with elongate apertures with these elongated at approximately right angles to apertural elongation of torted gastropods. The inhalant water current enters under the spire with the result that the "base" of the shell is frequently concave to accommodate the inhalant stream. Position of anus frequently marked by angulation or re-entrant at the outer part of upper whorl surface of aperture. Pretorsional left gill presumably lost.

Stratigraphic distribution—Low. Camb. - Dev. ?Perm.

Order ORTHOSTROPHINA Linsley & Kier, new order

Diagnosis—Orthostrophic paragastropods with either round or elongated apertures.

Stratigraphic distribution—Low. Camb. - Mid. Camb.

Superfamily Pelagiellacea Knight, 1956

Diagnosis—Characters same as order.

Stratigraphic distribution—Low. Camb. - Mid. Camb.

Family Pelagiellidae Knight, 1956

Diagnosis—Orthostrophic, right-handed paragastropods with an elongated tangential aperture. Inhalant current entering near umbilical area, frequently marked by a sinus. Exhalant current exiting near periphery of shell and marked by angulation or even a selenizone generating sinus. Shell rather flattened on top and arched below.

Stratigraphic distribution—Low. Camb. - Mid. Camb.

Genera included—Pelagiella Matthew, 1895; *Cambretina* Horný, 1964; *Costipelagiella* Horný, 1964; *Proeccyliopterus* Kobayashi, 1939.

Family ? Aldanellidae Linsley & Kier, new family

Diagnosis—Orthostrophic, right-handed paragastropods with a round, tangential aperture.

We have not been able to judge whether these shells have a tangential aperture.If they do, then they may well be mollusks because the tangential aperture implies a dorsally situated shell. If they have a radial aperture, then we suspect that they are not mollusks. Since aldanellids have a circular aperture we cannot state definitely that they are untorted, but their geological position would suggest that relating them to the penecontemporaneous pelagiellids is a more reasonable approach than suggesting that they are related to gastropods which do not appear until the Upper Cambrian.

Stratigraphic distribution—Low. Camb.

Genera included—Aldanella Vostokova, 1962; *Philoxenella* Vostokova, 1962; *Paraaldanella* Golubev, 1976; *Barskovia* Golubev, 1976.

Order HYPERSTROPHINA Linsley & Kier, new order

Diagnosis—Paragastropods with hyperstrophic to depressed-orthostrophic shell, commonly with angulation on outer part of upper whorl surface marking the exhalant channel. Inhalant current entering the mantle cavity at or near umbilicus; long axis of aperture converging toward apex of depressed spire; shell wall thick, outer layers calcitic, inner layers thick, aragonitic but not nacreous; operculum heavy, calcareous, paucispiral in *Maclurites* with attachments for two retractor muscles, unknown in other genera; right ctenidium inferred to have been absent.

Stratigraphic distribution—?Mid. Camb., Up. Camb. - Dev., ?Up. Trias.

Superfamily Onychochilacea Koken, 1925

Diagnosis—Hyperstrophic shells with highly prosocline tangential apertures. Shell form varying from high-spired to moderatey low-spired.

Stratigraphic distribution—Up. Camb. - Dev.

Family Onychochilidae Koken, 1925

Diagnosis—The area of the depressed spire gently rounded into umbilical area with only gentle angulation to mark exhalant area.

Stratigraphic distribution—?Mid. Camb., Up. Camb. - Dev.

Genera included—?Protoscaevogyra Kobayashi, 1939; *Matherella* Walcott, 1912; *Kobayashiella* Endo, 1937; *Matherellina* Kobayashi, 1933; *Pervertina* Horný, 1964; *Invertospira* Horný, 1964; *Helicotis* Koken, 1925; *Laeogyra* Perner, 1903; *Onychochilus* Lindström, 1884; *?Sinistracirsa* Cossmann, 1908; *Hyperstrophema* Horný, 1964; *Versispira* Perner, 1903; *Antispira* Perner, 1903.

Family Clisospiridae Miller, 1889

Diagnosis—The area of the depressed spire with sharp ridge on the upper whorl face.

Stratigraphic distribution—Ord. - Dev.

Subfamily Clisospirinae
Miller, 1889

Diagnosis—Low-spired forms with sharp ridge surrounding depressed spire at periphery and extended upwards and outwards as a frill.

Stratigraphic distribution—Ord. - Sil.

Genera included—Clisospira Billings, 1865; *Ferrogyra* Horný, 1964.

Subfamily Trochoclisinae
Horný, 1964 (emend.)

Diagnosis—Herein emended to refer to medium-spired conical clisospirids. Sharp angulation located at whorl periphery. Angulation may be extended upwards and outwards as a frill.

Stratigraphic distribution—Sil. - Dev.

Genera included—Conoclisa Horný, 1964; *Trochoclisa* Horný, 1964.

Subfamily Atracurinae
Horný, 1964 (emend.)

Diagnosis—Herein emended to refer to high-spired clisospirids. Sharp angulation located in from the periphery.

Stratigraphic distribution—Ord. - Dev.

Genera included—Mimospira Koken, 1925; *Antigyra* Horný, 1964; *Antizyga* Horný, 1964; *Atracura* Horný, 1964; *Bodospira* Wängberg-Eriksson, 1979; *Angulospira* Wängberg-Eriksson, 1979; *Tapinogyra* Wängberg-Eriksson, 1979; *Undospira* Wängberg-Eriksson, 1979.

Superfamily Macluritacea
Fischer, 1885

Diagnosis—Rather large, hyperstrophic shells with radial apertures. Aperture rather elongated with angulation at upper surface that is presumed excurrent. Base flattened or gently protruding.

Stratigraphic distribution—Up. Camb. - Ord.

Family Macluritidae
Fischer, 1885

Diagnosis—Same as Superfamily.

Stratigraphic Distribution—Up. Camb. - Ord.

Genera included—Scaevogyra Whitfield, 1878; *Palliseria* Wilson, 1924; *Maclurites* Lesueur, 1818; *Macluritella* Kirk, 1927; *Teiichispira* Yochelson & Jones, 1958.

? Superfamily Euomphalacea
de Koninck, 1881

Diagnosis—Shell mostly discoidal, orthostrophic or hyperstrophic; aperture round, radial, sometimes with angulation on upper whorl face, representing position of exhalant channel. Presumably with a single gill.

Stratigraphic distribution—Ord. - Perm., ?Up. Trias.

Family Euomphalidae
de Koninck, 1881

Diagnosis—Shell mostly discoidal, typically with wide umbilicus; abandoned early part of whorls closed off by septa; disjunct coiling common.

Stratigraphic distribution—Ord. - Perm., ?Up. Trias.

Genera included—Ophiletina Ulrich in Ulrich and Scofield, 1897; *Lytospira* Koken, 1896; *Lecanospira* Butts, 1926; *Barnesella* Bridge & Cloud, 1947; *Euomphalopsis* Ulrich & Bridge, 1931; *Ecculiomphalus* Portlock, 1843; *Lesueurilla* Koken, 1898; *Poleumita* Clarke & Ruedemann, 1903; *Centrifugus* Bronn, 1834; *Sinutropis* Perner, 1903; *Straparrollus (Straparollus)* de Montfort, 1810; *S. (Euomphalus)* Sowerby, 1814, *S. (Serpulospira)* Cossmann, 1916; *Nevadaspira* Yochelson, 1971; *S. (Amphiscapha)* Knight, 1942; *S. (Leptomphalus)* Yochelson, 1956; *Pleuronotus* Hall, 1879; *Mastigospira* LaRocque, 1949; *Phanerotinus* Sowerby, 1844; *Cylicioscapha* Yochelson, 1956; *Planotectus* Yochelson, 1956; *Discotropis* Yochelson, 1956; *Austerum,* Heidecker, 1959; *Labrocuspis* Heidecker, 1959.

Family Omphalotrochidae
Knight, 1945

Diagnosis—Shell trochiform, with broad sinus in upper part of outer lip and forward protrusion below; narrowly to widely phaneromphalous, aperture radial.

Stratigraphic distribution—Dev. - M. Perm.

Genera included—Orecopia Knight, 1945; *Omphalotrochus* Meek, 1864; *Babylonites* Yochelson, 1956; *Diploconula* Yochelson, 1956.

Family Omphalocirridae
Linsley, 1978

Diagnosis—Large, discoidal shells, frequently with circumbilical keel; early whorls filled with septa at maturity; operculum disc-shaped, multispiral. Exhibit sexual dimorphism.

Stratigraphic distribution—?U. Sil., Dev.

Genera included—Hypomphalocirrus Linsley, 1978c; *Omphalocirrus* Ryckholt, 1860; *Liomphalus* Chapman, 1916.

Family Oriostomatidae
Wenz, 1938

Diagnosis—Closely coiled shells with radial apertures; with heavy multispiral calcareous operculum; shell with nacreous inner layer.

Stratigraphic distribution—Up. Sil. - L. Dev.

Genera included—Morphotropis Perner, 1903; *Beraunia* Knight, 1937; *Oriostoma* Munier-Chalmas, 1876.

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LITERATURE CITED

BATTEN, R. L., ROLLINS, H. B. & GOULD, S. J., 1966, Comments on "the adaptive significance of gastropod torsion." *Evolution*, 21: 405–406.

BILLINGS, E., 1865, *Paleozoic fossils.* Geological Survey of Canada, Montreal, 1: 1–426.

BRIDGE, J. & CLOUD, P. E., Jr., 1947, New gastropods and trilobites critical in the correlation of Lower Ordovician rocks. *American Journal of Science,* 245: 545–559, 2 pl.

BRONN, H.G., 1834, *Lethaea Geognostica, oder Abbildungen und Beschreibungen der für die Gebirgs-Formationen bezeichenensten Versteinerungen.* Stuttgart, 1: 1–37.

BUTTS, C., 1936, The Paleozoic rocks. In Geology of Alabama. *Geological Survey of Alabama, Special Report* 14: 41–230, pl. 3–76.

CHAPMAN, F., 1916, New or little-known Victorian fossils in the National Museum, pt. 19, The Yeringian gastropod fauna. *Proceedings of the Royal Society of Victoria,* n.s., 29: 75–102, pl. 2–6.

CLARKE, J. M. & RUEDEMANN, R., 1903, Guelph fauna in the State of New York. *New York State Museum Memoir* 5: 1–195, 21 pl.

COSSMANN, M., 1908, *Revue critique de paléozoologie,* 12: 1–68.

COSSMANN, M., 1916, *Essais de paléoconchologie comparée.* Paris, 10: 1–292, 12 pl.

COX, L. R., 1960, General characteristics of Gastropoda. *In* KNIGHT, J. B., *et al., Treatise on Invertebrate Paleontology,* Part I, Mollusca 1. Geological Society of America and University of Kansas, Boulder, Colorado and Lawrence, Kansas, xiii + 351 p.

CROFTS, D. R., 1937, The development of *Haliotis tuberculata,* with special reference to organogenesis during torsion. *Philosophical Transactions of the Royal Society of London,* ser. B, 228: 219–268.

CUVIER, G. L. C. F. D., 1979, *Tableau élémentaire de l'histoire naturelle des animaux.* Paris, 710 p., 14 pl.

EALES, N. B., 1950, Torsion in gastropods. *Proceedings of the Malacological Society of London,* 28: 53–61.

ENDO, R., 1937, Part III: Addenda to Parts I and II, in ENDO, R. & RESSER, C. E. The Sinian and Cambrian formations and fossils of southern Manchoukuo. *Manchurian Science Museum Bulletin,* 1: 302–369, pl.

FISCHER, P., 1885, *Manuel de conchyliologie et de paléontologie conchyliologique, ou histoire naturelle des mollusques vivants et fossiles,* (8): 689–784. Paris.

GARSTANG, W., 1928, The origin and evolution of larval forms. *Report of the British Association,* Glasgow, 1928. Section D: 77–98.

GHISELIN, M. T., 1966, The adaptive significance of gastropod torsion. *Evolution,* 20: 337–348.

GOLUBEV, S. N., 1976, Ontogenetic changes and evolutionary trends in early Cambrian spiral gastropods of the superfamiy Pelagiellacea. *Paleontological Journal,* 10: 143–149.

HALL, J., 1879, Containing descriptions of the Gastropoda, Pteropoda and Cephalopoda of the Upper Helderberg, Hamilton, Portage and Chemung Groups. *Natural History of New York.* Paleontology, 5(2): 1–492, 113 pl.

HEIDECKER, E., 1959, Middle Devonian molluscs from the Burdekin formation of North Queensland. *University of Queensland Papers. Department of Geology,* 1(2): 1–11.

HORNÝ, R., 1963, Lower Paleozoic Bellerophontina (Gastropoda) of Bohemia. *Sbornik Geologisckých věd Paleontologie řada* P. sv. 2, p. 57–164, 44 pl.

HORNÝ, R., 1964, Nové Rody Gastropodo z českého starsího Paleozoika (Mollusca). *Časopis Národního Muzea, Oddíl přírodovědný Ročník,* 133(4): 211–216, 2 pl.

KIRK, E., 1927, *Macluritella,* a new gastropod genus from the Lower Ordovician of Colorado. *American Journal of Science,* (5)14: 288–292.

KNIGHT, J. B., 1937, Genotype designations and new names for invalid homonyms among Paleozoic gastropod genera. *Journal of Paleontology,* 11: 709–714.

KNIGHT, J. B., 1942, Four new genera of Paleozoic Gastropoda. *Journal of Paleontology,* 16: 487–488.

KNIGHT, J. B., 1945, Some new genera of Paleozoic Gastropoda. *Journal of Paleontology,* 19: 573–587, pl. 79–80.

KNIGHT, J. B., 1952, Primitive fossil gastropods and their bearing on gastropod evolution. *Smithsonian Miscellaneous Collections,* 117(13): 1–56, pl. 1–2.

KNIGHT, J. B., 1956, New families of Gastropoda. *Journal of the Washington Academy of Science,* 46: 41–42.

KNIGHT, J. B., COX, L. R., KEEN, A. M., SMITH, A. G., BATTEN, R. L., YOCHELSON, E. L., LUDBROOK, N.H., ROBERTSON, R., YONGE, C. M. & MOORE, R. C., 1960, *Treatise on Invertebrate Paleontology, Part I, Mollusca 1.* Geological Society of America and University of Kansas, Boulder, Colorado, and Lawrence, Kansas, xxiii + 351 p.

KOBAYASHI, T., 1933, Faunal study of the Wanwanian (basal Ordovician) series with special notes on the Ribeiridae and the ellesmereoceroids. *Journal of the Faculty of Science, University of Tokyo,* sect. 2, 3: 249–328, 10 pl.

KOBAYASHI, T., 1939, Restudy of Lorenz's *Raphistoma broggeri* from Shantung with a note on *Pelagiella. Jubilee Publication in Commemoration of Professor H. Yabe's 60th birthday,* p. 283–288.

KOKEN, E., 1896, *Die Leitfossilien.* Leipzig, 848 p., 241 pl.

KOKEN, E., 1898, Ueber untersilurische Gastropoden. *Neues Jahrbuch für Mineralogie, Geologie, und Paleontologie,* 1: 1–251.

KOKEN, E., 1925, Die Gastropoden des baltischen Untersilurs. *Academic Science Russian Memoirs* (PERNER, J. ed.). Classe physico-mathematique, (8) 35(1): 1–326, 41 pl.

KONINCK, L. G. DE, 1881, Faune du calcaire carbonifère de la Belgique, 3^{e} partie, Gastéropodes: *Museum Royal d'Histoire Naturelle de Belgique, Ann. Ser. Paleontologie,* 6: 1–170, 24 pl.

LAROCQUE, A., 1949, New uncoiled gastropods from the Middle Devonian of Michigan and Manitoba: *Contribution Museum of Paleontology, University of Michigan* 7(7): 113–122, 1 pl.

LESUEUR, C. A., 1818, Observations on a new genus of fossil shells. *Journal of the Academy of Natural Sciences of Philadelphia,* 1: 310–313, pl. 13.

LINDSTRÖM, G., 1884, On the Silurian Gastropoda and Pteropoda of Gotland. *Kongliga Svenska Vetenskaps-Akademiens Handlingar,* 19(6): 1–250, pl. 1–21.

LINSLEY, R. M., 1977, Some "laws" of gastropod shell form. *Paleobiology,* 3: 196–206.

LINSLEY, R. M., 1978a, Shell form and the evolution of gastropods. *American Scientist,* 66(4): 432–441.

LINSLEY, R. M., 1978b, Locomotion rates and shell form in the Gastropoda. *Malacologia,* 17: 193–206.

LINSLEY, R. M., 1978c, The Omphalocirridae: a new family of Paleozoic Gastropoda which exhibits sexual dimorphism. *Memoirs of the National Museum of Victoria,* 39: 33–54.

LINSLEY, R. M., YOCHELSON, E. L. & ROHR, D. M., 1978, A reinterpretation of the mode of life of some Paleozoic frilled gastropods. *Lethaia,* 11: 105–112.

MATTHEW, G. F., 1895, The *Protolenus* fauna. *Transactions of the New York Academy of Science,* 14: 101–153, 11 pl.

MCNAIR, C. G., KIER, W. M., LCCROIX, P. D. & LINSLEY, R. M., 1981, The functional significance of aperture form in gastropods. *Lethaia,* 14: 63–70.

MEEK, F. B., 1864, Section 1. Description of the Carboniferous fossils. *In Geological Survey of California. Paleontology,* v. 1.

MILLER, S. A., 1889, *North American geology and paleontology.* Cincinnati, Ohio, Western Methodist Book Concern, 664 p.

MONTFORT, P. D. DE, 1810, *Conchyliologie systématique et classification méthodique des coquilles; offrant leurs figures, leur arrangement generique, leurs descriptions caracteristiques, leur noms; ainsi que leur synonymie en plusieurs langues.* Tome 1. *Coquilles univalves, non cloisonnées.* Paris.

MUNIER-CHALMAS, E., 1876, Mollusques nouveaux des terrains paléozoïques des environs de Rennes. *Journal de Conchyliologie,* 24: 102–109.

PEEL, J. S., 1974, Systematics, ontogeny and functional morphology of Silurian trilobed bellerophontacean gastropods. *Bulletin of the Geological Society, Denmark,* 23: 231–264.

PERNER, J., 1903, Patellidae et Bellerophontidae: in BARRANDÉ, J., *Système silurien du centre du la Bohème,* v. 4 Gastéropodes. Tome 1: 1–164, 89 pl.

PORTLOCK, J. E., 1843, *Report on the geology of the county of Londonderry and of parts of Tyrone and Fermanagh.* Dublin, 748 p., 54 pl.

ROBERTSON, R. & MERRILL, A. S., 1963, Abnormal dextral hyperstrophy of post-larval *Heliacus* (Gastropoda: Architectonicidae). *Veliger,* 6: 76–79.

ROLLINS, H. B. & BATTEN, R. L., 1968, A sinus-bearing monoplacophoran and its role in the classification of primitive molluscs. *Palaeontology,* 11: 132–140.

RUNNEGAR, B. & POJETA, J., Jr., 1974, Molluscan phylogeny: the paleontological viewpoint. *Science,* 186: 311–317.

RYCKHOLT, [P.] DE, 1860, Revue des genres qui

composent la famille des Haliotidae, d'Orbigny. *Journal de Conchyliologie,* 8: 183–188.

SMITH, F. G. W., 1935, The development of *Patella vulgata. Philosophical Transactions of the Royal Society of London,* 225: 95–125.

SOWERBY, J., 1814, *Nos. IX and X in the mineral conchology of Great Britain; or colored figures and descriptions of those remains of testaceous animals or shells, which have been preserved at various times and depths in the earth* (in Vol. 1). London.

SOWERBY, J., 1884, *No. CX of the mineral conchology of Great Britain; or colored figures and descriptions of those remains of testaceous animals or shells which have been preserved at various times and depths of the earth* (a part of vol. 2). London.

THOMPSON, T. E., 1967, Adaptive significance of gastropod torsion. *Malacologia,* 5: 423–430.

ULRICH, E. U. & BRIDGE, J., 1931, Description of species—Gastropoda, in ULRICH, E. O., FOERSTE, A. F. & BRIDGE, J.: Systematic paleontology, in BRIDGE, J., *Geology of the Eminence and Cardareva quadrangles.* Missouri Bureau of Geology and Mines, (2)24: 186–207, pl. 18, 21–22.

ULRICH, E. O. & SCOFIELD, W. H., 1897, The Lower Silurian Gastropoda of Minnesota. *Minnesota Geological Survey,* 3(2): 813–1081, pl. 61–82.

VALENTINE, J. M., 1979, How many phyla? The regulation of diversity in Metazoan grade and ground plan. *Geological Society of America,* 11(7): x. 531. Abstract.

VOSTOKOVA, V. A., 1962, Cambrian gastropods from the Siberian Platform and Tajmyr. *Nauchno-issledovatel'skii Institut Geologii Arktiki Ministerstva geologii i okhrany nebr SSSR, Sbornik,* 28: 51–74. In Russian.

WALCOTT, C. D., 1912, New York Potsdam-Hoyt fauna in Cambrian geology and paleontology. *Smithsonian Miscellaneous Collections,* 57(9): 251–304, pl. 37–49.

WÄNGBERG-ERIKSSON, K., 1979, Macluritacean gastropods from the Ordovician and Silurian of Sweden. *Sveriges Geologiska Undersökning,* ser. C, Nr. 758, Arsbok 72, Nr. 20: 1–33.

WENZ, W., 1938, Gastropoda. In SCHINDEWOLF, O. H. (ed.), *Handbuch der Paläozoologie, Band 6,* Teil 1: Allgemeiner Teil und Prosobranchia, viii + 240 p. Borntraeger, Berlin.

WHITFIELD, R. P., 1878, Preliminary descriptions of new species of fossils from the lower geological formations of Wisconsin. *Annual Report of the Wisconsin Geological Survey for 1877:* 50–89.

WILSON, A. E., 1924, A new genus and a new species of gastropod from the Upper Ordovician of British Columbia. *Canadian Field Naturalist,* 38: 150–151, 2 pl.

YOCHELSON, E. L., 1956, Permian Gastropoda of the Southwestern United States: 1. Euomphalacea, Trochonematacea, Pseudophoracea, Anomphalacea, Craspedostomatacea, Platyceratacea. *American Museum of Natural History Bulletin* 110: 173–276, pl. 9–24.

YOCHELSON, E. L., 1967, Quo vadis, Bellerophon? In TEICHERT, C. & YOCHELSON, E. L., eds., *Essays in Paleontology and Stratigraphy.* Kansas University Press, p. 141–161.

YOCHELSON, E. L., 1971, A new Upper Devonian gastropod and its bearing on the problems of open coiling and septation. *Smithsonian Contributions to Paleobiology* (3): 231–421, 2 pl.

YOCHELSON, E. L., 1978, An alternative approach to the interpretation of the phylogeny of ancient mollusks. *Malacologia,* 17: 165–191.

YOCHELSON, E. L. & JONES, C. R., 1968, *Teiichispira,* a new Early Ordovician gastropod genus. [U.S.] *Geological Survey Professional Papers* 613-B: 1–15, 2 pl.

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SPECULATIVE FUNCTIONAL MORPHOLOGY AND MORPHOLOGY THAT COULD NOT FUNCTION: THE EXAMPLE OF *HYOLITHES* AND *BICONULITES*

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ABSTRACT

Hyolitha have an elongate narrow shell, composed of calcium carbonate, that is closed posteriorly and open anteriorly. On the basis of my interpretation of their hard parts, the hyoliths are presumed to be an extinct class of mollusks containing three orders: Orthothecida, Hyolithida, and Toxeumorphida. Because of the inferred difficulty of movement, the Hyolitha are presumed to have been benthic organisms and probably detritus feeders. This concept is derived from various attempts to restore soft-part morphology in a plausible manner. Members of the Hyolithida might have been capable of pulling themselves along by using the pair of curious appendages at the aperture, but no such appendages are known for the other two orders. *Biconulites* is an Early Cambrian fossil that has been based on a post mortem agglomeration of hyolithid shells. No functional interpretation of *Biconulites* seems possible; there appears to be no workable combination of soft parts that could fit into the fossil remains. Similar assemblages of stacked tubelike fossils have been reported from post-Cambrian rocks.

INTRODUCTION

Any survey of functional morphology of the Mollusca should include both living and extinct forms. Most living Mollusca have a radula—though representatives of the class Bivalvia do not—and they have a mantle, although the mantle is not unique to the Mollusca. Except in the most remarkable specimens, the radula, as well as the soft organs, is not preserved in fossil mollusks, and the mantle is only represented by its end product of a calcium carbonate shell. Fossils are assigned to the mollusks because their shape generally resembles that of living forms and because the shell has a structure and composition that is, or is presumed to be, like that of the living taxa.

Geologically young fossils can be readily assigned to living groups, but the farther back in time one looks, the harder it is to be certain of taxonomic affinities of some fossils. The Hyolitha, extinct since the end of the Paleozoic, present an interesting case and show the kind of speculation one must indulge in when dealing with functional morphology of fossils. Except in the rarest of specimens, the paleontologist has no access to the soft parts. Yet, if fossils are to be considered as more than simply "nuts and bolts," some speculation must be undertaken. In the past century, hyoliths were assigned to the "Pteropoda" (Miller, 1899; see Yochelson, 1979a), for the shape of some of the shells included in the group was like that of some of the living Pteropoda. Subsequently, for many years hyoliths were virtually ignored as Mollusca. About three decades ago they were combined with other tubelike fossils into an extinct class, Coniconchia (Lyashenko, 1957; see Yochelson, 1961) and subsequently into a much better combination under the name Calyptoptomatida (Fisher, 1962). Fisher (1962: W116–W123) has given a summary of past classification and various interpretations of possible function of these fossils during their life. Data on their morphology and diversity has increased since that work, though these are still far from well known.

Within the last decade, Marek & Yochelson (1976) have suggested that Hyolitha may be an extinct class of Mollusca; alternatively, these fossils may be a separate extinct phylum (Runnegar *et al.*, 1975; Runnegar, 1980). Dzik (1978, 1980) has compared the larval stages (protoconch) of hyoliths to those of gastropods, citing this as further evidence of molluscan affinities; he has placed hyoliths under the Monoplacophora, although Hyolitha and Monoplacophora have quite different adult morphology. Many molluscan forms have a similar early larval development. Even if the fossils are not mollusks, they pose fascinating problems for a student of function-

al morphology and deserve to be more widely known. I continue to think that they are Mollusca, deserving of class status, though admittedly they are somewhat different from those classes that have living representatives conventionally assigned to the phylum.

CLASSIFICATION AND EXTERNAL MORPHOLOGY

The Hyolitha have a two-layered calcium carbonate shell showing cross-lamellar structure (Runnegar *et al.,* 1975), resembling that of other mollusks. The shell is closed at the apex, expands gradually, and develops an elongate form. Almost all hyolith shells are bilaterally symmetrical, but a few taxa have radial symmetry. If the Hyolitha are considered a class, three orders are recognized.

The Orthothecida (Fig. 1) are generally the smallest. The shell is straight; some members show radial symmetry, the only members in the class to do so, but most are oval to kidney shaped in cross section. An operculum is present, which apparently could be withdrawn into the shell for a short distance.

The Hyolithida (Fig. 2) are the most abundant and diverse of the Hyolitha.Shells range from 1 to 100 mm in length. Although some of the shells are straight, most are gently arched, following what seems to be logarithmic curvature at an extremely low angle. The longer side of the curve is the flattened anterior, and it extends forward to form a rounded shelf (ligula) in front of the aperture. Commonly, the cross section is triangular, but it may be modified to trapezohedral or ovoid. An operculum rests at the apertural opening and cannot be withdrawn. On either side, between the shell and the operculum, are two curved struts (helens) formed of calcium carbonate. These struts grow as a third hard part of the hyolithid skeleton (Yochelson, 1974). They are outward and posteriorward. Runnegar (1980) has suggested that the tips projected ventrally rather than dorsally, an interpretation with which I do not concur.

Finally, the Toxeumorphida Shimanskiy, an enigmatic group, has recently been assigned to the Hyolitha (Yochelson, 1979b; Peel & Yochelson, 1980). The shells of this order have a straight shell and broadly oval cross-section. They expand at a slower rate than do the Orthothecida and grow to an exceptionally large size. Details of the interior are not known, nor is it known whether they possessed a calcified operculum like that of the other two orders.

GENERAL CONSIDERATIONS

Because fossils are the remains of former living organisms, and not simply curiosities in the rock, they must have lived and reproduced. The modern biota gives us some notion of the living process and the various strategies that different forms pursue to survive. By judging from their hard parts that the Hyolitha were Mollusca, one also makes some inferences concerning their digestion and excretion, their respiration, and their circulation. One then can use some of the general features of Mollusca to interpret what the soft parts of these extinct organisms may have been like. This is a fundamental point. If these fossils were assumed to be "worm tubes," a different set of assumptions about soft parts would be used. If they were placed in yet another phylum represented by living organisms, or in a totally extinct phylum, still other assumptions would probably come into play.

The confirmation of an elaborately folded gut in the orthothecids (Thoral, 1935; Marek, 1967) was given by Runnegar *et al.* (1975) as

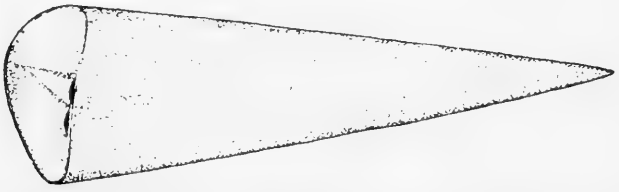

FIG. 1. Reconstruction of an orthothecid conch with operculum in situ, showing a pair of slots between the operculum and the ventral apertural margin of the conch, about three times natural size. From Marek, 1967, fig. 7, by permission.

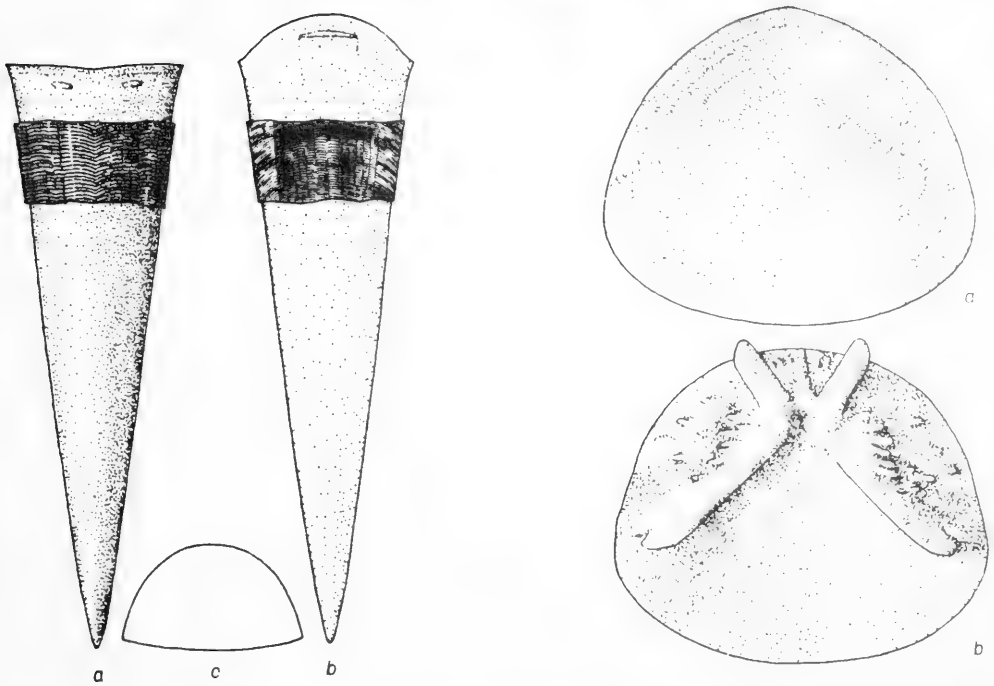

FIG. 2. A representative hyolithid, *Joachimites modestus* Marek, from the Upper Ordovician of Czechoslovakia. Conch in dorsal, ventral, and cross-sectional views, about one-third natural size; operculum in exterior and interior views about natural size. From Marek, 1967, figs. 15 and 16, by permission.

one of their reasons for suggesting that the Hyolitha are an extinct phylum. These workers also interpreted a serial repetition of muscles within the anterior part of the shell.

Independently, Marek & Yochelson (1976) worked on the biology of the Hyolitha; the later publication of their paper provided them the opportunity to comment on the work of Runnegar *et al.* Marek & Yochelson noted that only one genus of the Hyolithida shows multiple scars in the conch and that these scars need not be an indication of multiple muscles but rather may represent only a change in position of a single set of muscles as the animal grew (see also Marek, 1967); Marek (written communication, 1981), has found muscle tracks for the dorsal apertural muscle on a Devonian conch, indicating migration of muscles associated with ontogenetic change of the operculum shape. Marek & Yochelson also noted that the presence of an elaborately folded gut in the Orthothecida did not necessarily mean that the Hyolithida had the same sort of intestine. Their reconstruction of soft parts in the Hyolithida is presented here (Fig. 3). The various inferences on which it is based are detailed in Marek & Yochelson (1976), as well as in earlier references to work on the morphology of the Hyolitha.

The struts ("whiskers" or helens) are known only from about half a dozen localities, yet the paleontologist presumes that all members of the order Hyolithida—though not all Hyolitha—possessed them. The long folded intestine of the Orthothecida can be demonstrated from four localities (France, Antarctica and two localities in Czechoslovakia, one of which is described according to a written communication from Marek in 1981), yet again the same assumption is made. Generalizations from such limited data are hazardous, but to wait for specimens of each genus that show the one-in-a-million example of remarkable preservation would make the progress of interpretation even slower. A functional morphologist who deals with fossils must recognize that generalization from so few data "is a crooked wheel, but it is the only game in town."

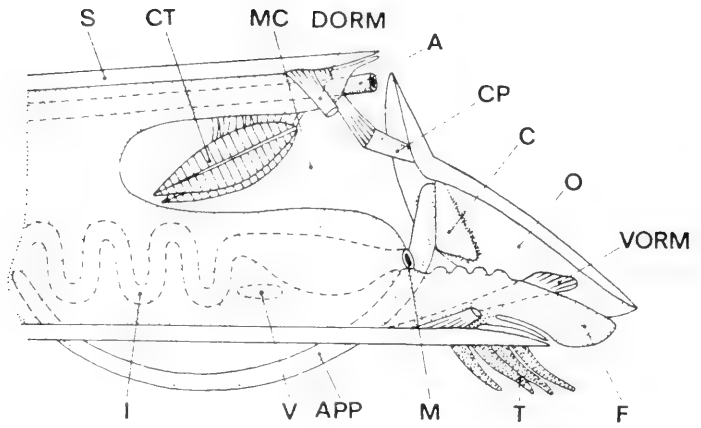

FIG. 3. Semidiagrammatic sagittal section through anterior part of a hyolithid with soft parts restored and operculum open, about six times natural size. The inner surface of the operculum was probably covered by tissue, but this is not shown. Lateral muscles and various visceral muscles associated with movement of fluid in vesicles also are not shown. The mantle folds at dorsal and ventral apertural margins are overemphasized deliberately and are not stippled for clarity. Tentacles are shown on only one side. Foot tentacles and main visceral mass are stippled but undifferentiated by distinct patterns. The operculum shows only cardinal process and clavicle on left side. The intestine contained many loops, as might be expected in a detrital feeder, but these are generalized and widened for simplicity in drawing. In several species of orthothecids, this organ is in a horizontal plane; for ease of drawing the hyolithid intestine is shown in a vertical plane, but there is no evidence to support either a vertical or horizontal configuration. A, anus; APP, appendage; C, clavicle; CP, cardinal process; CT, paired ctenidia; DORM, dorsal opercular retractor muscle; F, foot; I, intestine; M, mouth; MC, mantle cavity; O, operculum; S, shell wall; T, tentacles; V, vesicle; VORM, ventral opercular retractor muscle. From Marek & Yochelson, 1976, fig. 3, by permission.

THE OPERCULUM AND THE QUESTION OF MOVEMENT IN THE HYOLITHA

In spite of the lack of torsion in the Hyolitha, as indicated by the bilaterally symmetrical shell, the Gastropoda may provide a better basis for interpreting the soft parts of Hyolitha than any of the other living classes. Gastropoda have an operculum, though it is seldom calcified and preserved as a fossil. Two of the three orders of Hyolitha definitely have calcified opercula, and the record of opercula for this class is better than that for fossil Gastropoda. Presumably the operculum served the function of protecting soft tissue from harm.

In the Orthothecida, the operculum probably was slightly smaller than the aperture and could have been withdrawn, perhaps one-quarter of the length, into the tubelike shell. The sealing of the conch by the operculum fortuitously preserved the filling, giving a replica of the interior of the folded intestine. Clearly, the soft parts must have been considerably compressed when the operculum retracted. One could argue that this ability might also have indicated the ability to expand and to extend the soft parts some distance beyond the aperture. To reason by comparison, living scaphopods have the ability to retract a fairly long distance into the tube. The scaphopod foot, though small, can be extended enough to permit the animal to burrow. Thus, it seems logical to postulate that the orthothecoids may have had an extendable foot big enough to drag the shell, even if specific details of this organ cannot be given. Speed of movement was not a consideration with such an elongate shell. If the organism was a detritus feeder, movement may have been irregular and for very short distances at a time.

The Hyolithida also have a calcareous operculum, which, like that of the Orthothecida, shows concentric growth. However, it fits snugly at the aperture and cannot be withdrawn. Because of the projecting shelf (ligula) on the ventral part of the shell, the operculum has a complex arched shape. On the inner surface, it contains muscle attachments. Even though the operculum is not hinged, some species have a ball and socket form of

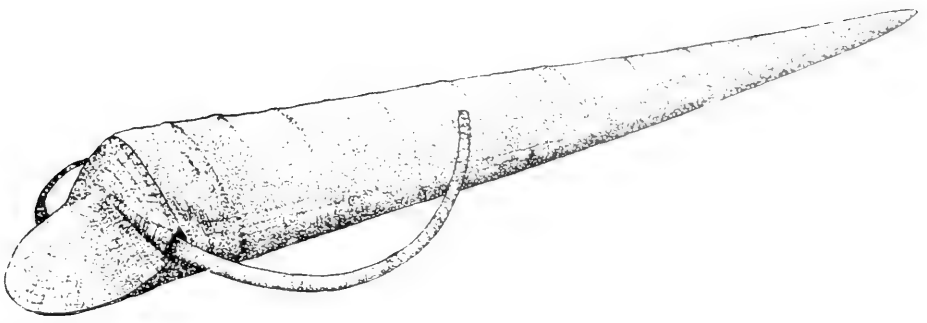

FIG. 4. Reconstruction of *Joachimilites novaki* Marek (1967), showing appendages in natural position with a closed operculum, about twice natural size. From Marek, 1963, fig. 15, by permission.

articulation at the dorsal angle. The helens fit snugly within the aperture against the inner surface of the operculum so that only limited movement was possible. The most logical use postulated for these structures is that of stabilizing the position of the shell on the bottom, much like the outriggers of a Polynesian canoe (Fig. 4). There may have been some movement when the operculum was closed that allowed the animal to move the shell forward by poling against the substrate, but the limited space between the inner edge of these structures and the inner surface of the operculum would have made for restricted, awkward, and inefficient movement at best. It is simpler to assume that food was brought to the vicinity of the shell by periodic currents, rather than *vice versa*, and that if the animal occasionally needed to mine fresh areas of the sea floor, the foot could extend far enough over the anterior shelf to jerk the shell forward. The large size of shells relative to the small size of the foot in some high-spired gastropods provides a parallel. Neither hyolithids nor orthothecids would have been particularly graceful, but the latter were more mobile.

No operculum is known for the Toxeumorphida. The shell is oval in cross-section but so close to circular that it is difficult to establish which side is dorsal and which is ventral. The rate of shell expansion is intermediate between that of the Orthothecida and the Hyolithida. Shells are large and may be more than 50 cm long. They rank among the largest of late Paleozoic invertebrates. Such animals must have been benthic and probably sedentary. They may have lived in areas of rich productivity, where food came to them without movement of the shell. Given the size and presumed weight of the shell and contained soft parts, it is hard to envision this form moving very far, for the foot would not increase in size as rapidly as the bulk of the body mass.

On the other hand, Dzik (1980) has compared some hyolithids with *Turritella*. He suggested that they had a filter-feeding mode of life and that the struts may have served to support inhalant siphons. The evidence is weak, to put it in the mildest terms. To me it seems unlikely that one of the oldest of mollusks could have developed filter feeding.

THE ORIGINAL DESCRIPTION OF *BICONULITES*

Teilhard de Chardin (1931) described the genus *Biconulites* from a single species, *B. grabaui,* found in the Early Cambrian of the southern part of Shansi Province, China; the species occurs in several beds. He considered *Biconulites* a "pteropod-like" organism. *Hyolithes* was classified at that time as a pteropod. Though the author recognized some resemblances between *Hyolithes* and *Biconulites*, he chose to interpret his form as a different organism. He may have been impressed with septa in the apical part of the shell, but these septa occur in several classes of mollusks, as well as in undoubted Hyolithida (Zazvorka, 1928).

According to its author, *Biconulites* had several hard parts (Figs. 5–6). The principal hard part was a "direct cone," slightly curved and septate in the apical area. Some specimens showed one or more other direct cones external to this one; there was no evidence of "fusion" between the shells of these cones.

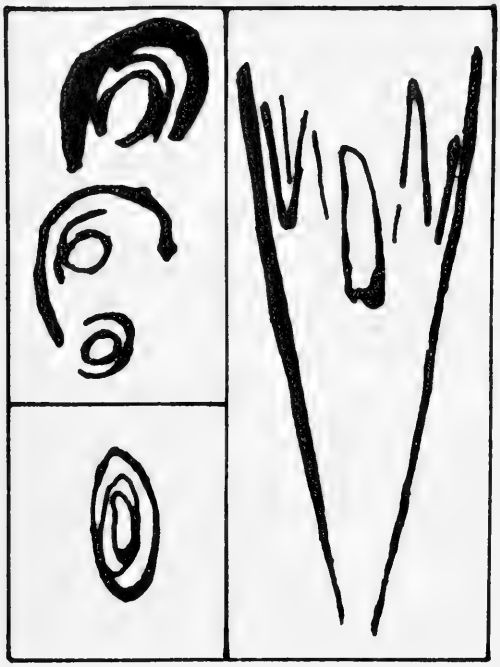

FIG. 5. *Biconulites grabaui* Teilhard de Chardin. Ink rendition of shell material in thin sections. Upper left pl. 1, fig. 6, and lower left, pl. 2, fig. 4, both showing transverse sections; right, pl. 2, fig. 5, showing longitudinal sections. All three times natural size. Modified from Teilhard de Chardin, 1931.

FIG. 6. *Biconulites grabaui* Teilhard de Chardin. Ink rendition of thin section showing transverse, cross and oblique section, three times natural size. Modified from Teilhard de Chardin, 1931, pl. 2, fig. 6.

Another part was an "inverted cone." This hard part had a different shape and an open apex; the inverted cone was symmetrical relative to the direct cone. A few specimens showed more than one inverted cone in the aperture of the direct cone. Finally, there was a "central tube." The only description of this is "The central tube is a very obscure feature, distinctly recognizable in a small number of specimens" (Teilhard de Chardin, 1931: 183).

Teilhard de Chardin noted two points in favor of the zoological uniqueness of these fossils—the striking geometric regularity and the consistency of complexity. Against the interpretation of these fossils as animals, he remarked that localization of the soft parts within the several direct cones seemed almost impossible and that shells might be "serrated and inserted into each other." He felt that the inverted cones were part of the organism, much like an operculum, but that the accessory inverted cones were more difficult to explain. His illustration of a dozen thin sections do not demonstrate to me either regularity or consistency for the organism.

LATER WORK ON *BICONULITES*

Both Spath (1936) and Kobayashi (1937), specialists on fossil mollusks, pointed out that *Biconulites* could be an artificial assemblage. In particular, Spath showed the great diversity among individuals and the obvious telescoping of several hyolith conchs in Australian representatives of *"Salterella" hartmanni* Foord from the Lower Cambrian.

In connection with a study of the rare Middle Ordovician conical orthothecoid *Polylopia* Clarke, Yochelson (1968) also figured a thin section of *B. hartmanni* (Foord) of Australia. One of the points of Yochelson's work on *Polylopia* was to note that the presumed multiple shell layers, comparable with the several direct cones of *Biconulites*, were variable

among individuals and constituted a post-mortem feature attributed to movement of shells by currents. The telescoping of one scaphopod shell into another had also been described from a Permian beach environment (Yochelson & Fraser, 1973).

Fisher (1962) placed *Biconulites* in a group of phylum, class, order, and family uncertain. He made several pertinent observations based on Teilhard de Chardin's figures. "No trace of fusion is seen between the central direct cone. Some shells show up to 4 inverted cones that act as 'opercula.' The geometric axis of the inverted cones does not correspond to that of the direct cones but runs obliquely to it. Inverted cones are always loose; central tube obscured" (Fisher, 1962: W138). Because hypoliths are curved and because the cross section is commonly bilaterally symmetrical rather than radial, it is sometimes difficult to interpret thin sections. The observation by Fisher that the axis of inverted cones is oblique to the axis of the main shell is exceedingly pertinent. If smaller shells were jumbled into the cavity of a larger shell, one should not expect symmetrical relations. It is yet more proof that the generic name *Biconulites* was based on material that was an assemblage of unrelated hard parts.

Notwithstanding Fisher's analysis, a major paper on *Biconulites* (Termier & Termier, 1971) described another species, *B. courtessolei*, from the Lower Cambrian of southern France. The authors considered the possibility of secondary accumulation of shells after death but rejected it. By making a comparison with the Early Cambrian fossil *Salterella*—then thought by these workers to be a cephalopod—and with younger fossil cephalopods, they deduced that *Biconulites* was somehow related to the cephalopods.

They concluded (Termier & Termier, 1971: 353–354, translation):

> The presence of a system of partitions and the presence of an endosiphon in *Salterella* and the biconulitids (central part), as well as in certain hyolithids induces us to believe that among the conical shells of the Lower Cambrian there was some tendency toward the style of cephalopods, tendencies which appeared distinctly earlier than the true phragmocone. The biconulitids (central part) have a number of points in common with the hyolithids, in particular probably the presence of an operculum in some of them. However, they are not comparable to other more specialized hyolithids which are very characteristically differentiated; they are close only to the circothecids with which they share a subcircular section and a generally arcuate form.
>
> Concerning the external cones, the hypothesis of a random imbrication remains the most plausible but we may also suggest that at least for some of them, the pallial epithelium may have secreted them to serve as cuticular envelopes which also may sometimes be agglutinated.

To try to clarify this summary a bit, *Salterella* is a small, radially symmetrical Early Cambrian fossil; it is about the size and shape of a pencil point. Laminae of tiny grains are placed within the cone. Loss of the shell and differential weathering of the grains gives a superficial resemblance to septa, and the genus was thought to be a primitive cephalopod for many years. (See Yochelson, 1977, for proposal of an extinct phylum for this form.) Within the last decade, the suggestion has been made that cephalopods are derived from an arched monoplacophoran mollusk that had multiple septa in the apical area (Yochelson, Flower & Webers, 1973). *Salterella* aside, the earliest cephalopods are in the Late Cambrian, some 50 million years later than the age of *Biconulites* described by Termier & Termier.

To the best of my knowledge, no analysis has been published elsewhere that compares *Biconulites* with cephalopods. The central structures, whatever they may be, are unlike the siphuncle of cephalopods. The siphuncle of a cephalopod pierces the septa and is never found in the living chamber. Those *Biconulites* that show septa have solid septal walls without any indication of a siphuncle. Furthermore, the hard parts taken to be allied to the siphuncle are in front of the septa!

How the several hard parts of *Biconulites* were "agglutinated," that is, stuck together by the action of an epithelium is also not quite clear. An alternative translation is "agglutinating," presumably indicating an all-encompassing epithelium that laid down an outer covering of small particles that is no longer preserved. By a vivid use of the imagination, one might describe *Xenophora* and a few other fossil gastropods that attach foreign particles to the shell (Linsley & Yochelson, 1973) as agglutinated, but that concept hardly applied to mollusks.

FIG. 7. Some hyolith conchs and some "*Biconulites*" from Hoppin Hill Reservoir, North Attleboro, Mass. About three times natural size. Drawn from a thin-section. USNM 317511.

CURRENT STUDIES OF *BICONULITES*

A limited amount of new data on *Biconulites* is available. I have observed the form in Lower Cambrian rocks at North Attleboro, Massachusetts (Fig. 7). The rock is crowded with authentic hyoliths and with trilobites, most of which are in disarticulated pieces. The sediments give some indirect evidence of currents, for the fossils are bunched in limited areas, not distributed uniformly along a bed.

Rock of similar age from Labrador has also been investigated. Again the rock is crowded with hyoliths but *Biconulites* is found only exceptionally (Fig. 8). These rocks were deposited in a quiet water environment (James & Debrenne, 1980), where it is much simpler to assume that a few shells have drifted together to form "*Biconulites*" than that most shells have been disarticulated to form hyolithids.

These two new occurrences fill in a gap in biogeographic distribution between Australia, China, and western Europe, if one considers *Biconulites* an organism. Far more importantly, they reinforce my opinion that *Biconulites* is nothing more than an artificial assemblage of shells. Indeed, I find the indication of transport in the original illustrations so overwhelming that I am baffled why the assemblage was ever formally named as a fossil. Even allowing for such bizarre recon-

FIG. 8. Assemblage of abundant hyolith conchs, trilobite fragments, and rare "*Biconulites*" from near Point Amour, Labrador. Drawn from a thin section, about twice natural size. USNM 317512.

structions that must be made for the single shells of hyolithids and orthothecids, no logical reconstruction could possibly fit the organs of respiration and digestion into the *Biconulites* shell. Variation among the assemblages aside, considerations of space show no way for a mantle to secrete the several parts and still form a mantle cavity in which other organs could function.

Biconulites probably includes both hyoliths and trilobite fragments. Certainly the hook-shaped and arched pieces of trilobite exoskeleton, seen in Figs. 4 and 8, are better suited to be "opercula" than are the elongate cones. An operculum ought to fit an aperture, and it is impossible to fit one cone to another of precisely the same width. If Teilhard de Chardin's types could be examined, one might be able to prove conclusively that more than one kind of organism is present.

Biconulites is a name in good nomenclatural standing. The provision for eliminating names based on "monsters" applies to abnormal growth, not artificial assemblages. Thus, the name cannot be removed from the literature. Sadly enough, although some geologists (Daily *et al.,* 1977: 20, fig. 11) recognize that the fossil record contains "hyolithid shell invaginated one into the other by current action," other geologists continue to use the name in faunal lists as though it had some biologic significance (Kruse & West, 1980).

Those *Biconulites* reported to date are of Early Cambrian age. Hyoliths are exceedingly abundant locally in the Early Cambrian and it makes sense that some of them might have been moved after death. My observations on the Canadian material suggest that it may not take vigorous wave or current action to imbricate hyolithids. The artificial assemblage need not be confined to rocks of one age, though post-Cambrian hyoliths are not normally found and are exceedingly rare in younger Paleozoic rocks. Accordingly, post-Cambrian *Biconulites* have not yet been found but may turn up at a few localities. We have in *Biconulites* a generic name with no particular age significance and no biological meaning.

SUMMARY

The hyoliths have a shell form not seen among living mollusks. Interpretation of their presumed functional morphology suggests that they were capable of, at best, limited movement. From the standpoint of biomechanics of living organisms, they were inefficient. However, hyoliths were abundant for 100 million years and persisted for about 300 million years.

Biconulites is an example of one of the pitfalls that face the paleontologist. Had even rudimentary consideration been given to the necessary organ systems for an animal, the name might never have been given to this artificial assemblage. The issue of disarticulation and artificial jumbling after death is very real to the vertebrate paleontologist, but many invertebrate paleontologists do not worry about it.

The imbrication and stacking of tubes by currents needs investigation. Although fossil scaphopod tubes have been stacked within each other by currents, I am not aware of any examples of scaphopods from present-day environments being so emplaced; to find some in the Holocene might be illuminating. The same kind of phenomenon leads to differential sorting of right and left valves of pelecypods and the occasional stacking of isolated shells. Fortunately for the neontologist, living pelecypods are available for comparison, and, to my knowledge, no one has described a genus based on isolated nested shells. I hope no one ever will.

LITERATURE CITED

DAILY, B., FIRMAN, J. B., FORBES, B. G. & LINDSAY, J. M., 1977, Geology, p. 5–42. *In* TWIDALE, C. R., TYLER, M. J. & WEBB, B. P. (eds.), *Natural history of the Adelaide region.* Royal Society of South Australia, Adelaide, Australia.

DZIK, J., 1978, Larval development of hyoliths. *Lethaia,* 11: 293–299.

DZIK, J., 1980, Ontogeny of *Bactrotheca* and related hyoliths. *Geologiska Föreningens i Stockholm Förhandlingar,* 107: 223–233.

FISHER, D. N., 1962, Small conoidal shells of uncertain affinities, p. W98–W143. *In* MOORE, R. C. (ed.), *Treatise on invertebrate paleontology, part W Miscellanea.* Geological Society of America and University of Kansas Press, 259 p.

JAMES, N. & DEBRENNE, F., 1980, First regular archaecyaths from the northern Appalachians Forteau Formation, western Newfoundland. Canadian Journal of Earth Sciences, 17: 1609–1617.

KOBAYASHI, T., 1937, On *Salterella canulata* and its allies. *Japanese Journal of Geology and Geography,* 14: 173–183.

KRUSE, P. D. & WEST, P. W., 1980, Archaeocyatha of the Amadeus and Georgina

basins. *BMR Journal of Australian Geology and Geophysics,* 5: 165–181.

LINSLEY, R. M. & YOCHELSON, E. L., 1973, Devonian carrier shells (Euomphalidae) from North America and Germany. *United States Geological Survey Professional Paper,* 824: iii + 26 p., 6 pl.

LYASHENKO, G. P., 1957, [Coniconchia, a new class of extinct Mollusca]. *Akademia Nauk SSSR Leningrad Doklady,* 117: 1049–1052. [In Russian].

MAREK, L., 1963, New knowledge on the morphology of *Hyolithes. Sbornik Geologickyckych véd, paleontologie,* 1: 52–73.

MAREK, L., 1967, The Class Hyolitha in the Caradoc of Bohemia. *Sbornik Geologickyčych véd, paleontologie,* 9: 51–112.

MAREK, L. & YOCHELSON, E. L., 1976, Aspects of the biology of Hyolitha (Mollusca). *Lethaia,* 9: 65–82.

MILLER, S. A., 1889, *North American geology and palaeontology for the use of amateurs, students and scientists.* Western Methodist Book Concern, Cincinnati, Ohio.

PEEL, J. S. & YOCHELSON, E. L., 1980, Giant Mollusca (Hyolitha) from the Permian of East Greenland. *Grønlands Geologiske undersøgelse Rapport,* 100: 65–67.

RUNNEGAR, B., 1980, Hyolitha: Status of the phylum. *Lethaia,* 13: 21–25.

RUNNEGAR, B., POJETA, J., Jr., MORRIS, N. J., TAYLOR, J. D., TAYLOR, M. E. & MCCLUNG, G., 1975, Biology of the Hyolitha. *Lethaia,* 8: 181–191.

SPATH, L. F., 1936, So-called *Salterella* from the Cambrian of Australia. *Geological Magazine,* 73: 433–440.

TEILHARD DE CHARDIN, P., 1931, On an enigmatic pteropod-like fossil from the Lower Cambrian of southern Shansi, *Biconulites grabaui,* nov. gen. nov. sp. *Geological Society of China Bulletin,* 10: 179–184.

TERMIER, G. & TERMIER, H., 1971, II-Observations sur le genre *Biconulites* (Mollusques?, Hyolithoides), p. 349–355 *In* COURTESSOLE, R., TERMIER, G. & TERMIER, H., Le Cambrien inferieur terminal de Ferrals-les-Montagnes (Hérault). *Société d'Histoire Naturelle de Toulouse Bulletin,* 107: 339–356.

THORAL, M., 1935, *Contribution à l'étude paléontologique de l'Ordovicien Inférieur de la Montagne Noire et révision sommaire de la faune Cambrienne de la Montagne Noire.* 326 p., Montpelier.

YOCHELSON, E. L., 1961, Notes on the class Coniconchia. *Journal of Paleontology,* 35: 162–167.

YOCHELSON, E. L., 1968, On the nature of *Polylopia. United States Geological Survey Professional Paper,* 593-F: iii + 7 p., 1 pl.

YOCHELSON, E. L., 1974, Redescription of the Early Cambrian *Helenia bella* Walcott, an appendage of *Hyolithes. United States Geological Survey Journal of Research,* 2: 717–722.

YOCHELSON, E. L., 1977, Agmata, a proposed extinct phylum of Early Cambrian age. *Journal of Paleontology,* 51: 437–454.

YOCHELSON, E. L., 1979a, Early radiation of Mollusca and mollusc-like groups, p. 323–358. *In* HOUSE, M. R. (ed.), *The origin of major invertebrate groups.* Systematics Association Special volume 12. Academic Press, New York and London.

YOCHELSON, E. L., 1979b, An alternative approach to interpretation of phylogeny of early mollusks. *Malacologia,* 17: 165–191.

YOCHELSON, E. L. & FRASER, G. D., 1973, Interpretation of depositional environment in the Plympton Formation (Perian) southern Pequop Mountains, Nevada, from physical stratigraphy and a faunule. *United States Geological Survey Journal of Research,* 1: 19–32.

YOCHELSON, E. L., FLOWER, R. H. & WEBERS, G. F., 1973, The bearing of the new Late Cambrian monoplacophoran genus *Knightoconus* upon the origin of the Cephalopoda. *Lethaia,* 6: 275–309.

ZAZVORKA, V., 1928, Revision of the Hyolitha from d_γ. *Palaeontographia Bohemiae,* 13: 1–22.

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9. Supply between five and eight key (topic) words to go at the end of the Abstract.

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INTRODUCTION TO THE SECOND INTERNATIONAL SYMPOSIUM ON MOLLUSCAN GENETICS

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Two general lessons are learned from the two international symposia on molluscan genetics (ISMG) that have been held: 1) Mollusks are superb organisms for studying problems in evolution and testing hypotheses involving both population and evolutionary genetics. 2) Genetic studies of mollusks have reached a level of considerable sophistication, to the point that *Drosophila* workers and paradigms based on *Drosophila* research henceforth have no monopoly of the field.

Mollusks are well suited for genetic studies. As a result of over 600 million years of evolution one sees in the extant 100,000 species, organisms demonstrating amazing variation in morphological ground-plans, physiologies, habitat tolerances, and reproductive strategies ranging from obligatory outcrossing, protandry, and simultaneous hermaphroditism, to obligatory parthenogenesis. Added to these advantages mollusks are rather plant-like, i.e. usually of low vagility, common in numerous types of habitats, easily collected, often in adequate numbers for some, if not many purposes, readily marked for recapture, and sometimes amenable for culture and controlled breeding.

The first ISMG was an outgrowth of the molluscan ecogenetics group (U.K.) founded by English workers in 1960. On the occasion of the 20th anniversary of the founding of the group, J. S. Jones organized a truly international conference on molluscan genetics held 17–19 April 1980 in the Royal Free Hospital and rooms of the Linnean Society in London (1980, *Nature*, 209: 283–284). It should be pointed out, however, that the molluscan ecogenetics group was regionally and sporadically international from the beginning and had meetings in Paris and Groningen. The second ISMG was sponsored by the American Malacological Union from 20–22 July 1982 in New Orleans, Louisiana, U.S.A. (organized by G. M. Davis, The Academy of Natural Sciences of Philadelphia (A.N.S.P.) and L. R. Kraemer, President of AMU-82, University of Arkansas). The contributions of ISMG-II grouped into the following categories: ecogenetics, cytogenetics, population genetics based on allozyme analyses, and species recognition and breeding systems. A section on medical malacology bridged all categories. There were 27 contributions in all.

Ecogenetics: Thus far there are three instructive paradigms for understanding modes of land snail speciation, deployment, diversity, and ecogenetics, i.e. *Cepaea* (European), *Cerion* (Caribbean), and *Partula* (Pacific Islands). Only papers on *Cepaea* and *Partula* were presented at ISMG-II. Pioneering work on breeding *Cepaea* to determine the genetic basis of shell banding opened the way to explore the effects of natural selection on shell phenotypes. Now, some years later it is clear that selection is not simply due to visual predation or to climatic factors but that various selective forces are active on different populations and in different geographic areas. With this understanding there have been considerable advances in the past few years. It is now clear that historical effects, including man's past and present use of land, may influence current genotypes in populations of *Cepaea.* The need for, and justification of long-term surveys of populations (10, 50, or 100 years) has been elegantly demonstrated (Cameron, Birmingham University and Dillon, Bulmershe College, England).

A density-dependent population phenomenon was demonstrated in wild populations of *Cepaea nemoralis* (Carter and Ashdown, Portsmouth Polytechnic, England). Shell size is inversely proportional to density; increased density reduces juvenile activity and growth. Reduced adult shell size decreases fecundity. The possibility that snail mucus plays a role in interfering with population density and certain morph frequencies was raised. The lesson

[1]Supported in part by NSF grant DEB-8118963.

from Greenwood and Parkin (University of Dundee, Scotland and University of Nottingham, England) is that in contemplating reciprocal transplant experiments to assess factors influencing morph frequencies, many pairs of sites should be used with large numbers of snails present at each; the populations should be followed for longer than one year. It was concluded, however, that it would be more efficient to try to understand the selective factors acting on populations *in situ* and to test the significance of these factors under controlled conditions.

A paper submitted to Malacologia by Ramos (Madrid, Spain) was added to the proceedings of this symposium because it added considerably to the topic of *Cepaea* ecogenetics. The topic of Ramos' paper is polymorphism of *Cepaea nemoralis* in the Spanish Occidental Pyrenees.

In a pair of papers on *Partula* (by Murray, University of Virginia, U.S.A. and by Clark, University of Nottingham, England) it was shown that *Partula* has, as has *Cerion,* very low vagility and gene flow. By use of genetically marked individuals introduced into a population they found that after 10 years no original individuals were alive but the mean spread of the introduced genes was 1059 cm; the maximum distance was 27 m. They presented evidence suggesting that dextral coiling in some populations of *Partula suturalis* evolved as an isolating mechanism, a consequence of interaction with sinistral *Partula mooreana.* In *P. suturalis* direction of shell coiling is determined by the genotype of the mother; sinistral is dominant to dextral. Sacchi (Pavia, Italy) discussed the climatic-ecological conditions associated with the distributional patterns of *Cepaea nemoralis* and *C. vindobonensis* in north-eastern Italy.

Heller (Hebrew University, Jerusalem, Israel) presented a convincing argument that rodent predators were a selective force overriding selection for white shells in the desert where white shell morphs would be expected to be selected for as an anti-radiation device. The data suggested that variation in intensity of predation pressure caused variation in the distribution of shell color-banded morphs of *Theba pisana,* and by implication in many other species. For years, banding variation in *Theba pisana* has defied description; no genetic basis for the observed banding patterns has been demonstrated. Now, in a pair of papers by Cain and Cowie (University of Liverpool, England), these difficulties have largely been overcome. A number of morphs have been characterized, including banded forms with band three enhanced, others with band three reduced, and one with a line of dots at the lowest level of band three, but genetically in the unbanded class. Unlike *Cepaea,* there is considerable variation in the expression of most morphs, attributed to genetic modifiers. In *T. pisana,* random samples can show nearly continuous variation, a problem blocking previous workers from successfully scoring morphs (Cain). At Tenby, Wales, at the northern edge of its range, *T. pisana* is biennial in response to a short growing season. At Tenby, at least three loci occur with two alleles showing dominance at each, with epistasy permitting only four main phenotypes to appear. Morph frequencies differ at different sites but have not differed significantly over five years. Banded snails seem to be at an advantage towards the second year of the life cycle; drift cannot account for this trend (Cowie).

A significant and much welcomed new addition to ecogenetic studies is the marine prosobranch *Thais (=Nucella) emarginata* (Palmer, University of Alberta, Canada). This species, which produces egg capsules and crawl-away young, can be readily bred and reared in the laboratory. It ranges from the Bering Sea to southern California. In spite of restricted gene flow (marked individuals move <2 m/month, about 5 m in 12 months) widely separated populations readily interbreed, producing F_1 generations growing to adult size. Genetic divergence (allozyme data) is surprisingly low among populations. Shell color, banding and sculpture vary with morph frequencies controlled as follows: black dominant over orange and white; lack of banding dominant to banding. Spiral sculpture may be controlled by a single gene or linked genes. The importance of work with this wide-ranging marine snail cannot be stressed enough.

Cytogenetics: Molluscan cytogenetics has finally come into its own as demonstrated in a superb paper by Babrakzai (Babrakzai and Miller, Central Missouri State University and University of Arizona, U.S.A.). Beautifully clear karyotype work was demonstrated using up-to-date banding techniques to investigate parental and hybrid stock involving the land snails *Ashmunella proxima albicauda* and *A. lenticula.* The intermediate nature of the hybrid shell was demonstrated. In the hybrids, univalent, multivalent formation and evidence for translocations, inversions, and stickiness of meiotic chromosomes were observed. Difference of karyotypes was demonstrated

for the parental taxa. There were two major lessons from this paper: 1) One can obtain considerable karyotypic data bearing on genetic differences between closely related taxa when modern banding techniques are used. 2) One must be cautious in synonymizing land snail species in various ecological settings such as those very similar species of the arid Southwest, U.S.A.

Advanced karyotyping techniques including banding markers were used, in a preliminary survey, to demonstrate differences among taxa of marine mussels and oysters (Thiriot-Quiévreux, Villefranche-sur-mer, France). The possible hybrid origin of certain taxa of *Bulinus* and chromosomal evolution of *Biomphalaria* and *Bulinus* were discussed using karyotypic data (Goldman and LoVerde, Baylor College of Medicine and State University of New York, Buffalo, U.S.A.). These snail genera are of medical importance because some species and populations transmit species of the human blood parasite *Schistosoma. Biomphalaria* is karyotypically less variable at the diploid level than is *Bulinus.* While the authors argue that there are contrasting different rates of chromosomal evolution, no time of divergence or actual rate of change can be given. They do indicate that greater karyotypic diversity in *Bulinus* may be the result of *Bulinus* having smaller effective population sizes than does *Biomphalaria.*

Medical Malacology: There were additional papers to the one given on *Bulinus* and *Biomphalaria* in the field of medical malacology. The genetic relationship between snail age and susceptibility of *Biomphalaria glabrata* to infections with *Schistosoma mansoni* was shown to have four components: 1) nonsusceptible at any age; 2) juvenile susceptible, adult nonsusceptible; 3) susceptible at any age; 4) juvenile susceptible, adult variable (Richards, Biomedical Research Institute, Maryland, U.S.A.).

These components are under oligogenetic control with a suggestion that some snails nonsusceptible at any age carry unexpressed the alleles for adult susceptibility. Some of the data gave rise to questions of whether nongenetic factors were involved.

Phenotypic diversity among populations of *Bulinus* on Mauritius prompted morphological, breeding, and molecular genetic studies of various populations (Rollinson and Wright, British Museum (Natural History), England). Their data indicate that one species is involved, with all populations susceptible to infection with *Schistosoma haematobium.* Allozyme data indicate regional differences in gene frequencies and high levels of heterozygosity. However, as they point out, the work is preliminary as insufficient loci have been examined to determine genetic distances among taxa.

The most sophisticated population genetic analysis of a molluscan group of medical importance involved *Biomphalaria glabrata* (Mulvey and Vrijenhoek, 1981, *Biochemical Genetics*, 19: 1169–1182). Some 10 laboratory-maintained populations of *B. glabrata* were analyzed electrophoretically for 28 loci, of which 16 were polymorphic. Crosses between strains were used to demonstrate the genetic basis of inheritance of the electromorph patterns. In their present paper, eight linkage groups based on 11 polymorphic loci were discerned through controlled crosses between populations. Considerable genetic distance was seen between some population pairs; one could entertain the idea of distinct species in a *B. glabrata* species complex. Little evidence for inbreeding of local populations in nature was found; strong differentiation among local populations could be attributed to low migration rates and restricted gene flow and/or to selection.

Population Genetics: In addition to the paper given by Mulvey, there were six involving population genetics. Five were concerned with marine bivalves. The most unresolved problem to come from these papers is that of considerable heterozygote deficiency (Hdf). In experiments with *Mercenaria,* clams of known genotype from wild populations were individually induced to spawn; all gametes were mixed at one time to produce a randomly bred cohort. The genotype frequencies of the cohorts differed significantly from Hardy-Weinberg expectation; only in the Lap locus was there a heterozygote deficiency. There was an association of some genotypes with shell size. Differential survival was invoked to explain the data (Adamkewicz, Taub and Wall, George Mason University, Virginia, U.S.A.).

Singh and Green (University of Western Ontario, Canada) took up the issue of Hdf. In molluscan species thus far studied where Hdf has been noted *(Crassostrea* [also Hedgecock, this symposium], *Littorina, Macoma, Modiolus,* and *Mytilus)* the species are outbreeding and the presence of many null alleles in all the randomly selected polymorphic loci is not likely. The same situation also occurs in shipworms (Bivalvia) and *Crepidula* (protandrous hermaphrodite gas-

tropods) independent of whether the species has planktonic larvae or not (Hoagland, personal communication). Singh and Green found that Hdf was dependent on age and stage of development in *Crassostrea, Macoma,* and *Mytilus.* The degree of homozygosity was negatively correlated with growth rate and metabolic efficiency, and slow growers had a higher post-settlement mortality rate. These results cannot be explained by the Wahlund effect, but do fit an hypothesis of balancing selection where different fitness is based on the stage of development.

Zouros and Foltz (Dalhousie University, Nova Scotia, Canada) presented three models to account for Hdf. Two invoked selection while a third, the favored one involving genotype-dependent spawning time, did not. Over-dominance for fecundity would enhance the effect of genotype-dependent spawning. One discussant placed selection last in considering explanations while A. J. Cain argued that selection and all other mechanisms should be considered equally; however if models not involving selection produce patterns mimicking the effects of selection, one should be extremely careful not to assume selection. It was pointed out by various discussants that much greater selection does occur in the wild than is generally appreciated.

Ten oyster populations from Chesapeake Bay were studied to determine amounts of genetic differentiation among them (Buroker, Rutgers University, New Jersey, U.S.A.). Genetic similarities were on the average 0.99. Statistical differences among demes involving 23 of 41 alleles were claimed. It was suggested that there were four sub-populations, but there was considerable discussion about the appropriateness of some of the statistical tools used. The final paper in this section involved populations of the freshwater prosobranch *Goniobasis proxima* (Dillon, A.N.S.P., U.S.A.). Multivariate analytic methods were used to support the conclusion that a number of measured anatomical structures had more inferred genetic component than others, and that population divergence measurements correlate both with geographic distance and environmental differences among populations.

Systematics and Evolution: Seven papers (including the *Bulinus* paper by Rollinson and Wright) assessed differences between taxa. Allozyme data played a key role in understanding patterns of species divergence and the extent of divergence. These papers readily fit into the context of a conference held at York, England by the Systematics Association (U.K.) (13–15 July 1982) on "Adaptation and Taxonomic Significance of Protein Variation." This was a timely conference as sufficient work in this area has been done to allow for a number of general statements to be made. 1) Molecular genetic data are generally of immense value for detecting patterns of divergence among taxa; they have served especially well in finding sibling species. 2) One must provide a solid species concept for the group of organisms being studied based on biological properties of the group in addition to molecular genetic data. One should not define species solely on allozyme data. It is clear that in certain instances allozyme data clearly indicate that one is dealing with a previously undetected species and in such instances one could define the newly discovered species on the basis of allozyme data alone. This is more the case in dealing with sympatric taxa than with allopatric taxa. However, even in these restricted instances, one should determine the divergent biological properties that are associated with the molecular genetic differences. 3) Compilations from the literature show that nearly all conspecific populations have a Nei's identity (I) of $\geq$0.90, congeneric species have I ranging from about 0.20 to 0.99. When allopatric congeneric species have high I, then molecular genetic data are of little value for assessing differences among species. 4) A published range of I or Nei's genetic distance D values indicating species or subspecies rank in one group does not necessarily hold for another group of organisms. 5) Electromorphs demonstrated in allozyme analyses are associated with environmental-physiological adaptation, i.e. they are under selection and not neutral; they are not associated with a random walk through nature. 6) Convergence is continually ignored or underestimated. There can be significant electromorph convergence just as there is considerable convergence of morphological character-states in group after group examined. If two data sets (e.g. allozymes and morphology) are not congruent, one should consider convergence in one or both of the data sets. The use of two or more types of data sets is encouraged, to test for problems of convergence or inadequacies of one type of data. 7) A universal molecular clock is a myth and cannot be supported by fact.

In the present symposium, conspecific pop-

ulations of oysters (three species) were separated by D of 0.1 while between-species D was about 0.6 (Hedgecock and Okazaki, University of California, Bodega Bay, U.S.A.). Allozyme data were used to demonstrate the genetic relationships among oyster populations and species. Hoagland (A.N.S.P., U.S.A.) used allozyme data to examine the possibility that populations of *Crepidula* in Florida, phenotypically like populations in New England, were sibling species. Observed differences in egg type and larval development led to the hypothesis that two sibling species existed. Several populations of five species from New England and California, U.S.A. and Brasil were studied in addition to populations of the two suspected sibling species. Intraspecific differences (Nei's D) ranged from 0.003–0.081. Between known species, D was >0.6; for sibling species, D was between 0.3 and 0.6. Many loci were diagnostic and considerable genetic divergence between New England and Florida populations was shown; the Floridian taxa are probably good species, based on reproduction and allozyme patterns.

Allozyme data were used to demonstrate massive convergence, sibling species, and relationships among taxa of different clades (Davis, A.N.S.P., U.S.A.). In this study, 39 populations of 24 + species of eight genera of Unionidae (freshwater bivalves) were analyzed, aided by multivariate methods involving multidimensional scaling, Prim network, and ordination diagrams. Genetic divergence among species of *Uniomerus* was of the same magnitude as that among species of *Elliptio*. Parallel evolution was demonstrated since there were three divergent clades of lanceolate-shaped *Elliptio* involving several species, not just two species as often stated in the literature. Sibling species were demonstrated in *Uniomerus* and *Elliptio*. One species relegated to *Fusconaia* was shown to belong to another genus.

The relationship between the mussels *Mytilus galloprovincialis* and *M. edulis* was reviewed (Gosling, Regional Technical College, Galway, Ireland). Here is a situation ideally suited for studies involving subspeciation or its reversal, i.e. intergradation of subspecies. A controversy revolves around whether the taxa are good species or one variable species. Molecular and cytological data indicate a significant amount of divergence. However, in the northern limit of *M. galloprovincialis* there appears, in some localities, to be complete intergradation while further south there are varying amounts of hydridization between taxa. In the north the two types seem to prefer different habitats, i.e. sheltered vs. exposed shores. Is this a case of subspecies coalescing as *M. galloprovincialis* spreads north from the Mediterranean Sea? Or is this a case where in more southerly regions there are subspecies that hybridize whenever the two taxa are in contact while in the north there is one species with two morphotypes and slightly different genotypes reflecting adaptation to the two different habitat types? Do the two morphotypes converge on the morphologies of the two southern subspecies?

Work on population structure of slugs begun in Selander's laboratory continues. Nine arionid and seven limacid species of slugs from Europe were studied in relationship to breeding (Foltz, Ochman and Selander, University of Rochester, New York, U.S.A.). This work ought to be as much involved in slug systematics as it is in populations genetics. The authors suggested that self-fertilization is less in limacid slugs than in arionids. Further, facultative selfing slugs presumably have higher levels of allele frequency heterogeneity than do outcrossing species. In discussion of this paper considerable focus was on identification of the species, because some results ran counter to others' experiences in breeding slugs. While it is certainly true that there are species complexes (based on allozyme data) where once only one species was recognized, it is also true that some species can only be identified on the basis of internal anatomical detail. It did not help the discussion to be told that slugs were difficult to identify because they lacked shells, that no internal anatomical structures were examined, and setting aside voucher specimens had not been considered.

Finally, as had been stressed at York, description of allopatric species and subspecies solely on allozyme data can rarely be justified although allozyme data are often instrumental in causing one to look for additional species-distinguishing characters. When sympatric taxa are studied, diagnostic loci, population genetic analyses of deviations of genotype frequencies from Hardy-Weinberg equilibrium expectations, and maximum likelihood analyses of many loci should be used to assess the significance of any difference between taxa (Chambers, Office of Endangered Species, Washington, D.C., U.S.A.).

All but three of the papers presented in the Symposium are published here.

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HABITAT STABILITY, POPULATION HISTORIES AND PATTERNS OF VARIATION IN *CEPAEA*

R. A. D. Cameron[1] & P. J. Dillon[2]

ABSTRACT

Recently, it has been suggested that patterns of variation in *Cepaea* populations relate to habitat stability and population history, and in particular that the microgeographical variation known as area effects is associated with habitat instability and population bottlenecks. In this study two districts in Wiltshire, England, with well documented landscape history were sampled to test predictions made of the types of variation to be found. Both areas contain stable and unstable habitats. In stable areas *Cepaea* shows variation with habitat of a type suggesting visual selection for crypsis. Less stable areas also show the variation, but to a lesser extent, and involving fewer loci. They also show area effects. The predictions are therefore substantially confirmed.

Spatial correlations in morph-frequencies are generally stronger in unstable areas than in stable ones, indicating stronger geographical patterns. The extent to which populations of *Cepaea* which have colonized downland woods match their habitat is dependent on distance from the nearest ancient woodland. These results strengthen the hypothesis that founder effect and other aspects of population history have an important role in determining the patterns of variation seen in many *Cepaea* populations. They are discussed in the light of previous work on area effects and similar phenomena both in *Cepaea* and in other organisms.

Key words: variation; founder effect; habitat stability; *Cepaea*.

INTRODUCTION

The microgeographical variation known as 'area effects' in the shell polymorphism of the land snail *Cepaea* has attracted much interest, and been attributed to many causes (Cain & Currey, 1963a,b,c; Goodhart, 1963; Arnold, 1971; Jones, Leith & Rawlings, 1977; Clarke, Arthur, Horsley & Parkin, 1978), and has been involved in discussions of sympatric and parapatric speciation (White, 1978). The role of founder effect, and other aspects of population history has been minimized by most workers (but see Goodhart, 1963). Recently, Cameron, Carter & Palles-Clark (1980) suggested that explanations involving founder effect and other aspects of population history might be the most convincing, since area effects are found, for the most part, on downland areas where previous grazing regimes would have restricted *Cepaea* to small isolated populations, with local extinctions and recolonization. Wright (1978) has suggested that such situations can give rise to area effects. Areas permitting more stable and continuous populations of *Cepaea* show patterns of variation associated with habitat or topography, which are more readily interpreted in terms of present environmental selection.

This general association of area effects and downland, and the interpretation placed on it, can be further tested. In areas containing both downland and more stable habitats, with good and detailed records of landscape history, the patterns of variation to be found can be predicted, and these predictions tested by survey. Where the history of individual sites can be ascertained, the association can be examined more closely than before, and variation in downland populations related to likely sources of colonization. If pairs of adjacent samples of contrasting habitat can be made, two-factor analysis of variance can determine the extent to which position and habitat account for the variation, and pairwise correlations can be used to test the expectation that geographical pattern is more marked in downland sites (Jones, Selander & Schnell, 1980). This study reports on the application of these tests in two surveys of *Cepaea* in southern Wiltshire. Both areas were selected be-

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cause good historical records were known to exist, and because both were known to have a mixture of downland and more stable habitats.

SURVEY AREAS AND SITE HISTORIES

Methods

Descriptions of landscape and individual site histories have been compiled from both primary and secondary sources. Primary sources are records made at the time for which information is required. Many of them are unpublished, and are not given individually here. A list of such sources and their locations has been deposited in the National Lending Library, Boston Spa, United Kingdom. Principal primary sources include:

i) Ordnance Survey Maps 1:2500 first edition 1873, giving details of hedges, woods and uncultivated land. Later editions give details of subsequent changes.

ii) Enclosure Awards, recording details of enclosure by Parliamentary Act, often with detailed maps. Such awards date from the late eighteenth and early nineteenth centuries in these areas.

iii) Tithe Commutation surveys, carried out in the mid-nineteenth century prior to the abolition of tithes. These often include maps with details of land use.

iv) Andrews' and Drury's Map of Wiltshire (1773). This map, the first in the county using modern survey techniques, has a scale of approximately 2 inches to the mile (c. 1:31,000) and has been republished in facsimile (1952). Details of woodland are given but not field boundaries or land use.

v) Private estate maps with varying amounts of detail. Ones relevant to the area are of the nineteenth century.

vi) Saxon and Medieval place names (Glover, Mawer & Stenton, 1939) which can be used to identify woodland areas.

vii) Domesday Book (1086)—as interpreted by Finn (1967) and Darby (1977). Gives some evidence on woodland and land use in a general sense. Detailed analyses for Wiltshire are given by Morgan (1935) and Grundy (1939).

viii) Saxon Land Charters (tenth century) defining boundaries, often by reference to woods and hedges which sometimes remain identifiable. Relevant charters are analysed by Taylor (1964), and Grose (1947) examined botanical references in particular.

Secondary sources, works published later than the times to which these refer, and based on analysis of primary sources, are referred to in the usual way. For Whiteparish, the detailed study by Taylor (1967) is the key work; its existence determined the choice of Whiteparish as a study area. For Cranborne, Gough (1979) provides an analysis of woodland and downland history, and gives access to many primary and secondary sources.

In addition to the documentary evidence, some attention has been paid to field evidence—presences of archaeological sites, ditches and banks, and to botanical evidence. Some indication of the age and status of woods and hedges may be given by their botanical composition (Peterken, 1974; Pollard, Hooper & Moore, 1974; Cameron & Pannett, 1980), and in Cranborne Chase detailed botanical evidence is given by Gough (1979). In general, field, botanical and documentary evidence is congruent; where it is not, the documentary evidence has been used with a few exceptions mentioned below.

The classification of sites is explained in more detail below. There is, inevitably, an element of doubt about the classification of a few sites, due to gaps in the documentary evidence, and it is, therefore, worth mentioning that most of the site classification was done by P. J. D. prior to seeing the sample scores prepared by R. A. D. C.

Landscape Histories

The locations of the districts sampled are shown in Fig. 1. Both contain areas of one-time chalk downland with recent enclosures and plantations (a synopsis of the history of such areas is given by Cameron *et al.*, 1980) in which *Cepaea* populations would have been reduced at times to small relicts (Cameron, Williamson & Morgan-Huws, 1977). They also contain areas of stable mixed agriculture and ancient woodland. The histories of the two districts differ in detail, and are dealt with separately.

The Cranborne Chase district (Fig. 2) includes the downland scarps, high plateau and

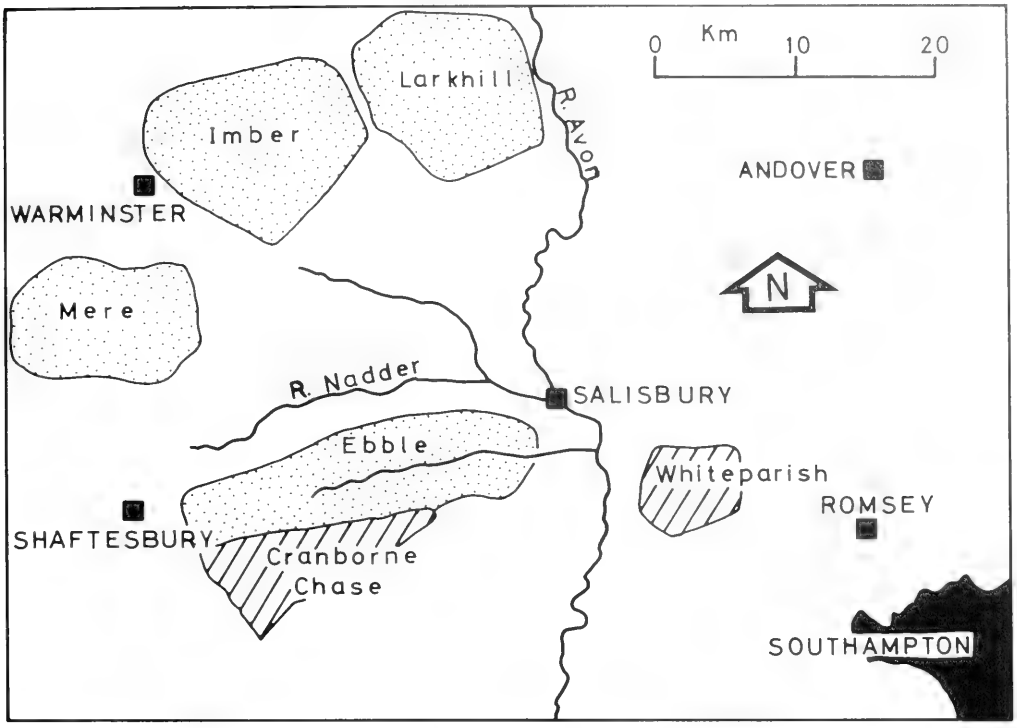

FIG. 1. Map showing location of study areas (hatched) and of others on Salisbury Plain (stippled) reported in Cain & Currey (1963b) and Cameron *et al.* (1980).

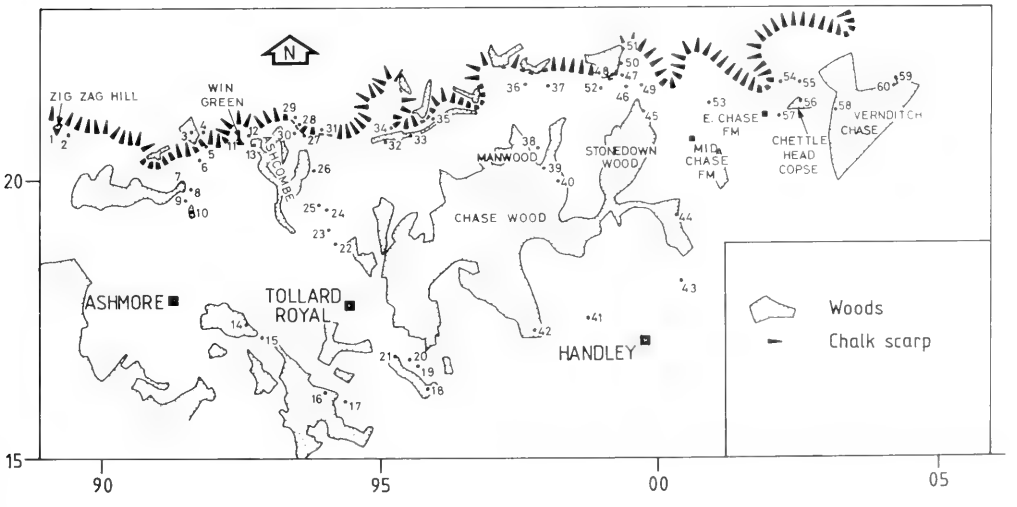

FIG. 2. The Cranborne Chase sampling area, showing the scarp, extant woodland and sampling sites. 5 km points of the National grid are shown in the margins.

some of the dip slopes to the south from Zig-Zag hill in the west to Vernditch Chase in the east. Many summits exceed 200 m., and the highest (Win Green) reaches 270 m.

The Chase differs from most chalklands in retaining a large extent of ancient woodlands on both scarp and dip slopes, due to its status as a private deer forest. This woodland is mostly coppice, and it has a continuous documentary history from the Middle Ages onward, while botanical evidence suggests that much of it is primary (Gough, 1979). The ancient woodlands of the scarp have been small and isolated for many hundreds of years, but the much larger woods of the dip slopes were continuous until the eighteenth and nineteenth centuries when some clearances were made (Lane-Poole, 1959; Andrews & Drury, 1773; Gough, 1979). The most conspicuous gap made in this way is around East and Middle Chase Farms.

Some of these woods cover prehistoric earthworks and barrows, and so not all are primary. They are, however, floristically rich (Gough, 1979), and since prehistoric clearance was often patchy and temporary (Thorley, 1981; Rackham, 1980; Cunliffe, 1973), they retain many primary characteristics. The present coppice management probably dates from Saxon times (Gough, 1979; see also Tittensor, 1980). The scarp woodlands, although ancient, are botanically rather poor, and may have been subject to occasional heavy grazing from sheep and rabbits from the surrounding open downland: in the dip slope woods only very restricted grazing by cattle was permitted (Gough, 1979). A number of ancient woodlands were extended by plantation in the nineteenth and twentieth centuries, notably East Combe Wood and Vernditch Chase.

The high plateau and most of the scarp have been sheep grazed from Bronze Age times until recently (Gough, 1979). In the last two centuries, a number of plantations and shelterbelts have been established, and most of the gentler slopes have been enclosed and converted to arable. Much of the remaining downland has become derelict, and given way to rough herbage and successional scrub woodland.

The Whiteparish area (Fig. 3) likewise includes a chalk scarp and the dip slopes to the south. The topography is more gentle than in Cranborne Chase, and altitudes are lower—the highest summits reach c 160 m. Dip slopes and plateaux have superficial non-chalky deposits in places, and the lower slopes have very acid soils on clays.

Taylor (1967) gives a very full account of the landscape history. In Saxon times, the scarps and parts of the plateaux were downland. Settlements in the central part of the dip slopes were surrounded by open fields, while the south remained heavily wooded. Woodland spurs also covered the dip slopes up to the summit on the western edge of the parish (adjoining Downton) and on the east to the summit at Mean Wood.

From Saxon times to the late eighteenth century there was a continuous process of clearance and improvement, coupled with a rise in population. Some woodlands were cleared and enclosed, the open fields disappeared under enclosure, and substantial areas of downland were brought under the plough. By the end of this period, large areas of woodland remained, but downland was largely restricted to the scarps, a few areas of summit, and a small area of dip slope on Pepperbox Hill.

Later developments include a number of plantations and avenues on the downland, planted extensions of existing ancient woods, and very recently the extension of scrub and rough herbage on the remaining downland as sheep and rabbit grazing declined. A number of ancient coppices have also been converted to conifer plantations, but retain a rich coppice fringe.

Individual Site Histories

Individual sites have been classified by their history, using the sources listed above. The main distinction used in this study is between sites with a downland history and those with a history of a more stable agricultural and woodland type. 'Downland' sites are those which have been grazed downland pasture at some stage in their history. Habitats in such sites include the successional stages from grassland to mature woodland, mostly rough herbage and scrubby woodland, and also hedges and plantation woodlands where it is clear that they were established within a downland area. 'Stable' sites do not have a history of use as downland pasture, and include ancient woods and hedges, and also later enclosure hedges and plantations in areas of mixed agriculture. Hedges and plantations made on downland but in direct contact with similar habitats in stable areas are, however, classed as stable, since they

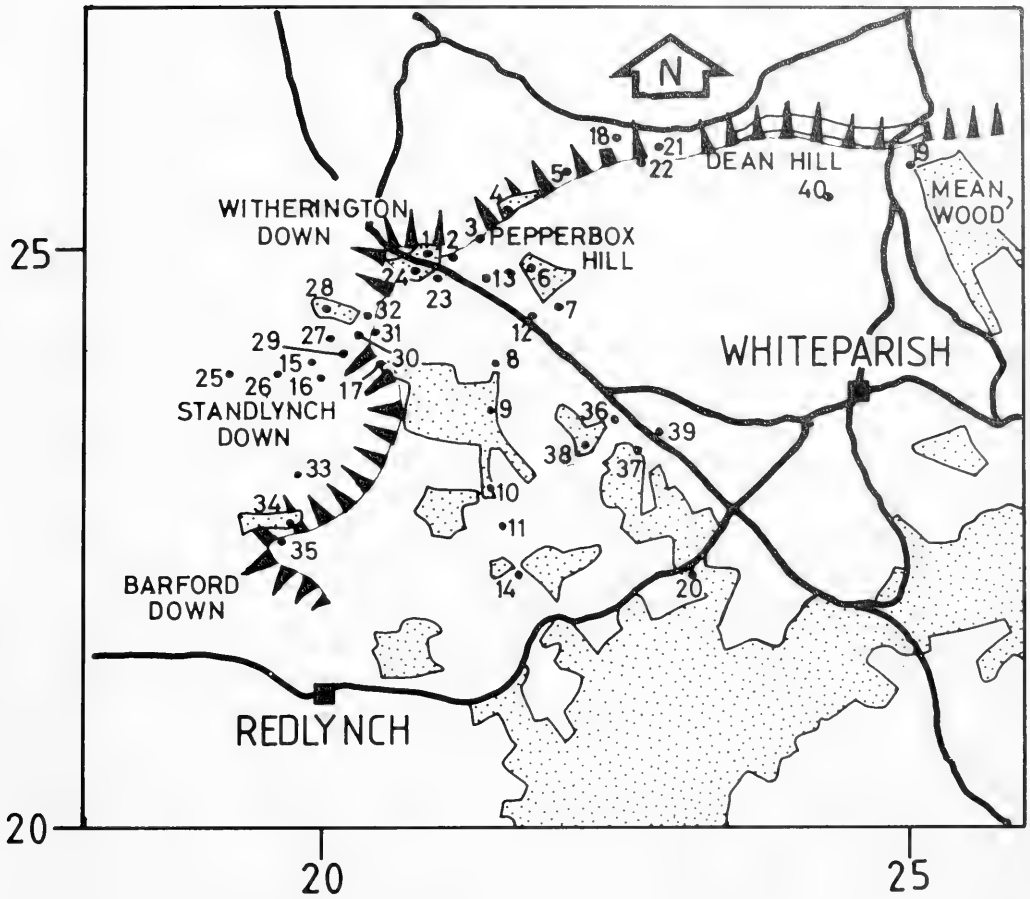

FIG. 3. The Whiteparish sampling area. Symbols as Fig. 2.

can be colonized directly without bottlenecks or changes in the visual selection regime.

Table 1 shows the classification of Cranborne Chase sites. Site 13 at Ashcombe presents some difficulty in classification. It comes from successional scrub woodland on the dip slope of Win Green, in a combe full of such woodlands. Evidence of grazing and scrub development from downland is abundant, and although now fenced, earlier maps show that this enclosure is a recent development. However, Andrews' and Drury's map shows enclosed woodland in the combe (although not on the sample site) and woodland in Ashcombe has even earlier references (Gough, 1979). The site has been classified as a downland wood.

Table 2 shows the classification of Whiteparish sites. As with Cranborne sites, this classification follows the documentary evidence, with only a few exceptions. Site 4, in Grimstead Beeches, does not appear as woodland on Andrews' and Drury's map, nor does wood appear to have been present in 1805. It is, accordingly, classed as a downland plantation, but is botanically anomalous, being much richer, and with a more complex age structure than other plantations on the plateau. Conversely, Site 6 on the dip slope of Pepperbox Hill is shown as woodland on Andrews' and Drury's map, but is botanically poor, with species characteristic of successional scrub. Its name, Upper Bushes, also suggests a scrub development, and it is classified as a downland site.

Sites 15, 16, 25–29 and 33 are presently in an area of arable farming developed by eighteenth/early nineteenth century enclo-

TABLE 1. Classification of Cranborne Chase sites.

(a) Stable habitats.		
Ancient woodland: 14, 16, 18, 39, 40, 42, 44, 45, 56. Extensions of ancient woodland: 21, 48, 50, 51, 58, 59. Plantations: 19.	}	woods
Hedges (mostly 18th Century): 15, 17, 20, 41, 43, 57.		open
(b) Downland habitats.		
Plantations: 1, 3, 7, 10, 11*, 23, 24, 26*, 28, 29, 33. Successional wood: 12, 13, 55*.	}	woods
Downland rough herbage: 2, 5*, 6, 8, 9, 25, 27, 30, 31, 34, 35, 36, 37, 38, 46, 47, 49, 52, 54*, 60. Isolated downland hedges: 4, 22, 32, 53.	}	open

*Sites containing *C. nemoralis* only.

TABLE 2. Classification of Whiteparish sites.

(a) Stable habitats.		
Ancient woodland: 9, 10, 20, 37, 38. Extensions of ancient woodland: 8, 17, 19. Plantations: 7, 15, 25.	}	woods
Old hedges: 11, 14, 36, 40. Enclosure hedges: 16, 26, 27, 29, 33, 39	}	open
(b) Downland habitats.		
Plantations: 1, 4, 21, 24, 28, 31, 34. Successional wood: 6.	}	woods
Downland rough herbage: 3, 12, 13*, 22, 23, 30, 32, 35. Isolated downland hedges: 2, 5*, 18.	}	open

*Sites containing *C. nemoralis* only.

sure and plantation. Part of this area may have been downland previously, but has had a large number of interconnecting stable habitats for two centuries, and all sites in it are classified as stable.

SAMPLING METHODS

Samples were made during the summer of 1981, using the techniques of Cain & Currey (1963a). Only samples containing twenty or more shells have been included in the analysis, except for a few samples of *C. nemoralis,* which is generally rare. In the Cranborne Chase area, some samples made in 1967–1971 along the northern edge of the survey area have also been included. Five of these were resampled; no significant differences or trends were found between the two occasions.

Habitats have been classified following Cain & Currey (1963a), but in general, the analysis concentrates on the differences between woods and all open habitats (in this case, hedges and rough herbage). In woods, some notes were taken on floral diversity and on the age structure and species composition of the trees as an aid to historical interpretation.

Wherever possible, samples were made in adjacent pairs, one from a wood, and one from an open habitat. In general, it was possible to make adequate samples within 600 m of each other. In a few cases, the distance exceeded 1 km, but the median distance between members of a pair is 300 m, a small distance relative to the sizes of the survey areas (15 × 6 km at Cranborne Chase, 6 × 4 km at Whiteparish). In a few cases, samples have no paired sample not already used in another pair. Where the distance is less than 1 km, these samples are paired with the nearest sample of contrasting habitat, but four rather isolated open habitat samples are left unpaired. Most pairs consist of samples with

similar landscape histories, but in some cases one member of a pair differs considerably from the other.

Jones, Leith & Rawlings (1977) give further details of the shell polymorphism and methods of scoring. In this study only fusions involving the upper three bands have been considered. The percentage of fusions is based on shells in which at least two of the top three bands are present, since the character can show itself only in such shells.

RESULTS

Cepaea hortensis

General Features of the Variation

C. hortensis is more abundant and widespread than *C. nemoralis,* and its variation is analysed in more detail. Full details of the samples are given in the Appendix. In both areas, *C. hortensis* is highly polymorphic for both colour and banding, and also shows considerable variation in the frequencies of fused-banded shells. Only a few samples are monomorphic for either colour or banding, but about a quarter of the samples from Cranborne lack fusions. There are slight overall differences between the two areas, Whiteparish having higher median values of yellow, unbanded and fusions.

Variation with Habitat

A number of patterns of variation with habitat consistent with visual selection for crypsis have been recorded for *Cepaea.* Of these, variation in yellow effectively unbanded appears unimportant in this study as frequencies of this combination are generally very low. Other patterns of variation concern yellow, unbanded and effectively unbanded, fusions in banded shells, and dark, the last category being a combination of all non-yellow effectively unbandeds and all banded shells with upper fusions.

Table 3 gives the median values for each of these categories by area, habitat and site history. Fig. 4 (A–D) shows the scatter of yellow and dark by area, site history and habitat, and Fig. 5 (A–D) shows fusions and effectively unbanded in the same way. In both areas, differences in medians between habitats are consistently greater, and in the direction expected if due to visual selection, in stable sites than in downland sites, where some variation is contrary to such expectation. The scatter diagrams show that in both areas, separation of habitats in morph-frequencies responsive to visual selection is much clearer in stable than in downland areas.

Because geographical variation may complicate analysis, statistical testing of this variation has been carried out by paired sample comparisons. The analysis is based on the status of the woodland member of each pair since there is evidence that visual selection is more effective there than in open habitats (Arnold, 1970, 1971; Cameron & Pannett, in prep.). Table 4 shows the results of paired sample comparisons, for each area separately, and for all samples combined, since in no case do the results from one area differ

TABLE 3. Median values of morph-frequencies (percent) in *C. hortensis* by habitat and site history.

	Stable woods	Stable open	Downland woods	Downland open
Cranborne Chase				
Yellow	43.0	73.0	78.1	68.0
Unbanded	29.0	17.7	20.0	14.3
Fusions	26.5	9.5	16.0	3.2
Effectively unbanded	35.7	17.7	25.0	20.7
Dark	45.5	15.8	23.0	11.8
Whiteparish				
Yellow	52.9	75.5	65.5	80.9
Unbanded	47.1	26.0	17.0	32.0
Fusions	52.9	25.0	34.8	14.3
Effectively unbanded	47.1	26.4	23.8	32.0
Dark	66.7	30.9	38.0	30.4

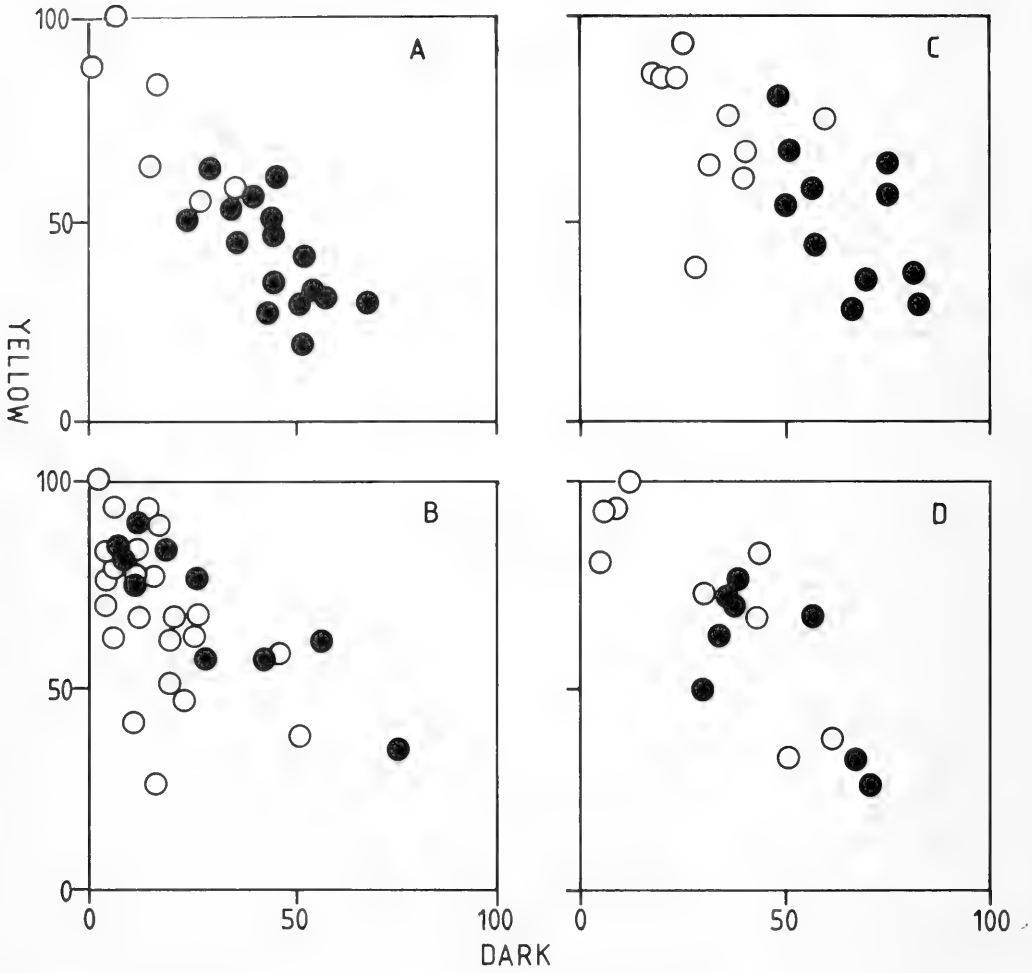

FIG. 4. Scatters of % yellow and % dark in *C. hortensis*. Solid circles, woodland samples; open circles, open habitat samples. A = Cranborne stable habitats, B = Cranborne downland habitats, C = Whiteparish stable habitats, D = Whiteparish downland habitats.

significantly from those in the other. In stable pairs, all results are consistent with variation due to visual selection, and all but two comparisons show significant departures from the null hypothesis that there are no differences between habitats. By contrast, in downland pairs, there are many fewer significant departures, and not all the trends are appropriate to a visual selection hypothesis. Only fusions and dark (which includes fusions) show consistent and significant trends of the sort seen in stable habitats.

Besides variation in individual morph-frequencies, variations in combinations and interactions may also respond to visual selection. Linkage disequilibria are generally either weak, or show the same bias in most samples, but between sample comparisons can also be made. Yellow and dark are consistently negatively associated, which is in part due to the fact that by definition dark includes all non yellow effectively unbanded shells. There are no other significant associations in open habitats. There are, however, some significant associations in woodlands, which can be compared with expectations derived from the hypothesis that downland populations are influenced by source of origin.

On the assumption that backgrounds in the

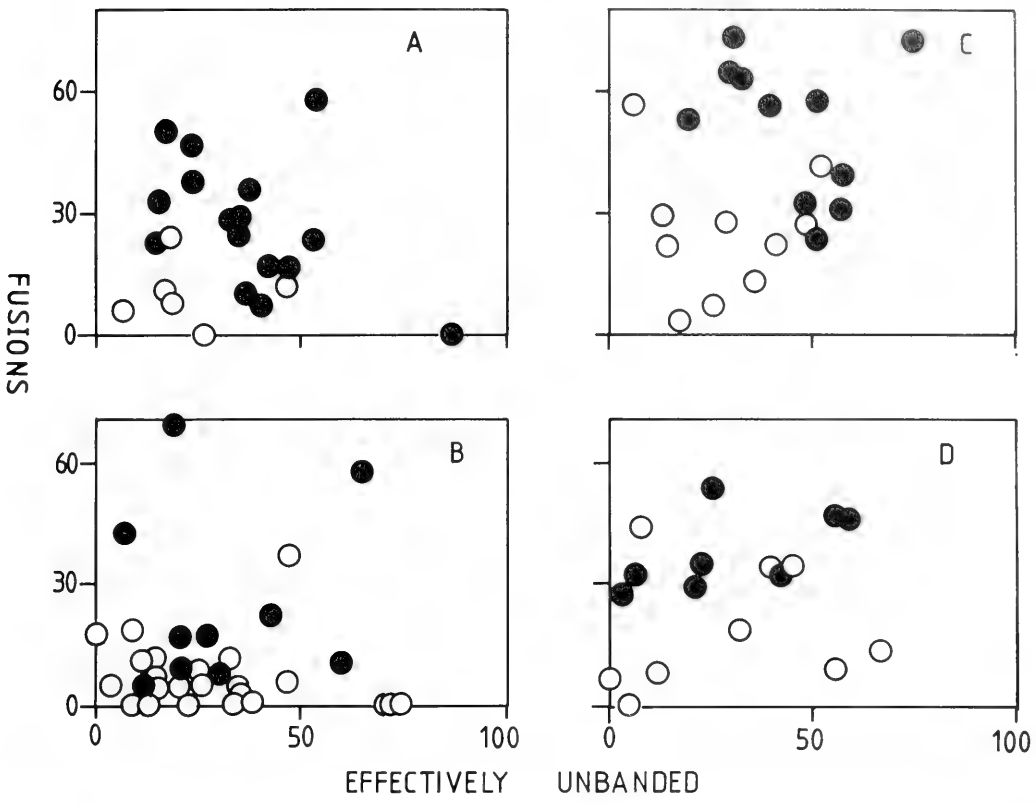

FIG. 5. Scatters of % fusions and % effectively unbanded in *C. hortensis*. Symbols and letters as Fig. 4.

TABLE 4. Numbers of sample pairs in which the morph listed is at a higher frequency in woodland (w) and in open (o). Ties omitted. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (binomial expansion or χ^2). In no case do results from Whiteparish and Cranborne differ significantly from each other.

	Whiteparish		Cranborne		Total	
	w	o	w	o	w	o
Stable woodland pairs						
Yellow	1	10**	0	9**	1	19***
Unbanded	10	1**	6	2^{ns}	16	3**
Fusions	11	0***	8	1*	19	1***
E.U.[1]	10	1**	7	2^{ns}	17	3**
Dark	11	0***	9	0**	20	0***
Downland woodland pairs						
Yellow	2	9^{ns}	7	6^{ns}	9	15^{ns}
Unbanded	4	7^{ns}	6	7^{ns}	10	14^{ns}
Fusions	8	3^{ns}	12	1**	20	4**
E.U.[1]	4	7^{ns}	6	7^{ns}	10	14^{ns}
Dark	8	3^{ns}	11	2*	19	5**

[1]E.U. = Effectively unbanded.

woodland sites are reasonably homogeneous, then the hypothesis above leads to different expectations in stable and downland woods. In stable woods a negative relationship between yellow and effectively unbanded is expected because yellow effectively unbandeds are very conspicuous. A negative association is also expected beween fusions and effectively unbanded, since these are the two components of dark:—if a given frequency of dark is appropriate to the background, then as one increases the other should decrease. In the case of yellow and fusions, a slight positive association is expected, since yellow shells are most conspicuous, and therefore benefit most from darkening due to band-fusion, but non yellow banded shells also benefit.

In downland woods, expectations generated by the hypothesis are somewhat different, if source of origin of the population has more importance. Yellow and effectively unbanded should be negatively associated, because sites colonized from open habitats will have high yellow and low effectively unbanded, and *vice versa* in those colonized from woodland. On the same argument, a positive association is expected between fusions and effectively unbanded, and a negative association between yellow and fusions.

Table 5 shows the relevant correlations. In no case do coefficients differ significantly between areas, and so overall comparisons via *z* transformation are also given (Fisher, 1944). Only a third of all associations are significant, but all of them are consistent with the expectations outlined above.

Microgeographical Variation

There are few clear-cut large area effects detectible by simple inspection of the data, but such inspection does suggest that adjacent samples often have similar morph-frequencies, especially on downland. The most striking and consistent example is to be found in Whiteparish, where downland samples fall into two groups—those associated with Pepperbox Hill, and those associated with Witherington and Standlynch Downs (see Fig. 3). The former have low frequencies of pink, brown, and unbanded, and the latter high ones, to the extent that populations from open downland resemble those from dark stable woods. Detailed analysis of microgeographical pattern has been carried out by pairwise and distance correlations of morph-frequencies, omitting effectively unbanded, because it is heavily dependent upon the frequencies of unbanded alone.

If downland populations are most influenced by historical factors, then within pair correlations should be greater in pairs with a downland component than in purely stable pairs. Further, distance between members of a pair should influence the correlation, short distances giving better correlations than long ones. The extent to which downland woods have morph-frequencies appropriate to stable woods will be a function of distance from the nearest stable wood from which colonizers might come.

Table 6 shows the relevant correlations. In Cranborne Chase samples, downland pairs show consistently stronger correlations than stable pairs for all morphs. In Whiteparish, however, this is true only of yellow; fusions and dark show no trends, while unbanded shows a very high correlation in stable pairs.

The effect of distance between members of a pair in downland areas has been analysed by calculating correlations separately for those pairs with less, and those with more than the median distance between their

TABLE 5. Correlation coefficients between morph frequencies in samples from stable and downland woods, based on arcsine transformed data. Overall correlations obtained by *z* transformation. n = number of samples. Probabilities as Table 4.

	n	Yellow and E.U.	Fusions and E.U.	Yellow and Fusions
Stable woods				
Cranborne	16	−0.2677	−0.5930*	+0.2738
Whiteparish	11	−0.7566**	−0.2150	+0.0363
Overall	27	−0.5005**	−0.4160*	+0.1832
Downland woods				
Cranborne	10	−0.0203	+0.0787	−0.7281*
Whiteparish	8	−0.4557	+0.6439	−0.3629
Overall	18	−0.2162	+0.3654	−0.6040*

members (200 m in each area). In all eight cases (four morphs in two areas) the 'close' pairs show a better correlation.

Morph-frequencies in downland woods have been correlated with the reciprocal of distance to nearest stable wood, which emphasizes local pattern by giving more weight to the shortest distances (Jones *et al.*, 1980). In both areas yellow increases, and fusions and dark decrease with distance from nearest old wood. Unbanded also decreases with distance in Whiteparish, where variation with habitat in this morph is marked, but not in Cranborne Chase, where such variation is minimal even in stable habitats (see Table 7 below).

Since both geographical and habitat variation are occurring together, the relative contribution of each to the overall variation in downland and stable areas should be examined. This has been done by two factor analysis of variance, using only pairs both members of which have the same kind of site history. Geographical variation is analysed by variation between pairs, rather than by any more subjective division into larger groupings (cf. Wright, 1978). The disadvantage of this procedure is that there are as many degrees of freedom between pairs as in the residual variance, and hence the proportion of variance accounted for by pairs is high even when there is no geographical effect. Table 7

TABLE 6. Correlation coefficients (i) between members of pairs in stable habitats, (ii) between members of pairs in downland habitats, overall, and for pairs with less (nearest) and more (furthest) than the median distance between their members, (iii) between frequency and the reciprocal of distance from nearest old woodland for downland wood samples. d.f. = degrees of freedom. $^{*}P < 0.05$, $^{**}P < 0.01$. Analysis of arcsine transformed data.

	(i) Stable pairs	(ii) Downland pairs			(iii) Downland woods distance from old wood (reciprocal)
		Overall	Nearest	Furthest	
d.f.	7	18	8	8	8
Cranborne					
Yellow	−0.1525	0.5441*	0.7350*	0.3372	−0.5563
Unbanded	0.0898	0.6538**	0.7699**	0.5155	−0.2872
Fusions	−0.4654	0.5782**	0.8469**	0.2369	0.8149**
Dark	−0.1950	0.5981**	0.8523**	0.0392	0.6622*
	7	11	5	4	6
Whiteparish					
Yellow	−0.2445	0.6910**	0.8362*	0.4803	−0.5353
Unbanded	0.8063**	0.4336	0.6614	0.1454	0.7658*
Fusions	0.2511	−0.0407	0.3784	−0.3484	0.4746
Dark	0.2420	0.2033	0.6090	−0.1456	0.7102*

TABLE 7. Percentage of total variation accounted for by habitats and pairs by area, morph, habitat and site history in two factor anovars. $^{*}P < 0.05$, $^{**}P < 0.01$. Analysis of arcsine transformed data.

	Yellow		Unbanded		Fusions	
	Stable	Downland	Stable	Downland	Stable	Downland
Cranborne						
Habitat	49.5**	1.0	7.9	0.1	47.7*	44.3**
Pairs	29.4	39.8	50.1	72.6*	14.1	52.6**
Whiteparish						
Habitat	43.3*	18.8*	22.1**	0.9	50.0**	35.2*
Pairs	21.4	64.0*	70.3**	86.0**	33.1	33.6

shows the variance accounted for by each factor. In all six cases, variation with habitat is greater in stable than in downland pairs, and variation between pairs is less. Unsurprisingly, the analysis confirms results described above: variation with habitat in downland is most marked in fusions; unbandeds show more variation with both habitat and pairs in stable habitats in Whiteparish than in Cranborne Chase.

Cepaea nemoralis

Details of samples are given in the Appendix. *C. nemoralis* is more localized than *C. hortensis,* and there are only a few samples for consideration. Formal analysis has therefore not been attempted, but a number of features of the variation are worth mentioning.

Cranborne Chase

The scatter of yellow and effectively unbanded is shown in Fig. 6, following Cain & Sheppard (1954). Samples from downland areas have more yellow and less effectively unbanded than those from stable areas. Within the downland category there is no difference between woods and open habitats on either axis. All samples from stable areas come from woods. Collectively, these occupy a space in the scatter diagram similar to that occupied by woodland samples in the Oxford district (Cain & Currey, 1963a). Unlike *C. hortensis,* planted or successional woodland populations adjacent to ancient woods show a deficiency in effectively unbandeds when compared with those from old woodland proper.

Geographical variation is most marked in the frequency of midbanded in banded (Fig. 7). There is a distinct area centered on the scarp summit at Win Green in which frequencies of midbanded are very low. Yellow and unbanded do not show the same marked pattern.

Whiteparish

There are only 10 samples, 9 of which come from the downland summit, and one from an ancient wood. Fig. 8 shows the yellow/effectively unbanded scatter. There is no evidence of variation with habitat, and the whole set of samples is characterized by extremely high frequencies of non yellow and midbanded shells. The one sample from ancient woodland is the least extreme for both these characters. The samples collectively appear to come from a classic area effect.

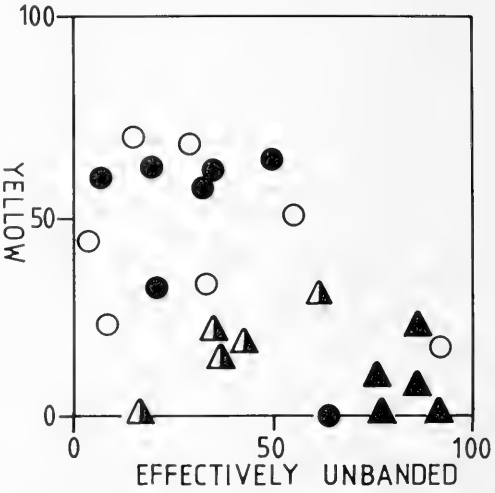

FIG. 6. Scatter of % yellow and % effectively unbanded in *C. nemoralis* in Cranborne. Solid circles, downland woods; open circles, downland open habitats. Solid triangles, ancient woods; half-filled triangles, plantation adjacent to ancient woods.

DISCUSSION

This study originated in the observation that area effects in *Cepaea* are frequently associated with areas of downland grass previously subject to heavy grazing by sheep and rabbits (Cameron *et al.,* 1980). When grazed, such areas are very marginal for *Cepaea* (Cameron *et al.,* 1977), an observation also made casually by Hudson (1900) who describes what appears to be an extreme five-banded area effect on downland, and contrasts it with the greater variety of morphs to be found elsewhere.

In the areas of mixed downland and stable habitats sampled at Cranborne Chase and Whiteparish, *Cepaea hortensis* populations show both variation with habitat, and with position. As predicted from earlier studies, microgeographical variation is strongest in downland habitats, and the relationship between the morph frequencies in downland woods and their distance from ancient woodland suggest strongly that source stocks have a persistent influence on daughter populations, a conclusion confirmed by the differing relationships between morphs seen in

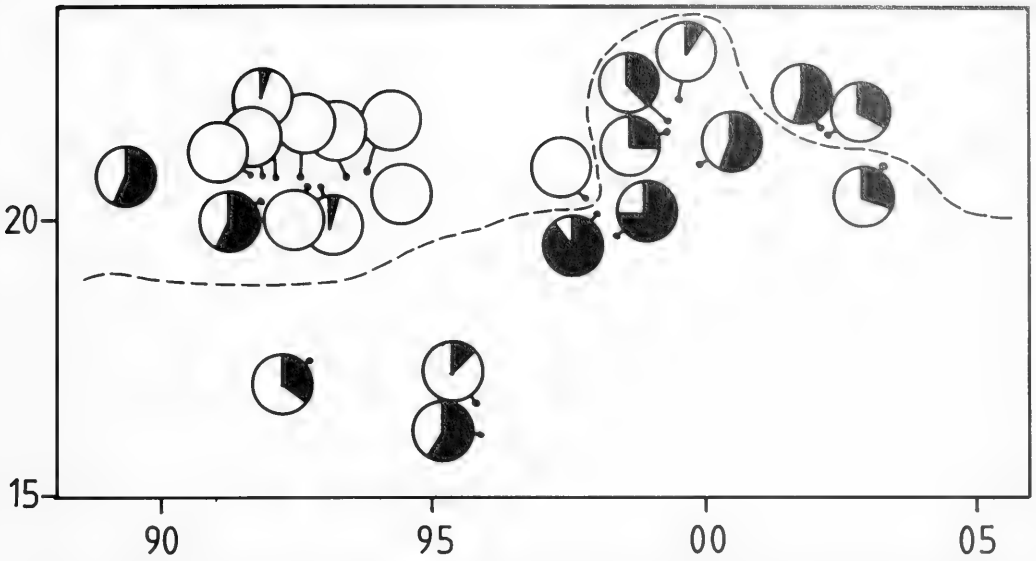

FIG. 7. Variation in % midbanded in banded in *C. nemoralis* in Cranborne. % midbanded represented by black portion of each circle. The dashed line separates downland (above) from stable (below) sites.

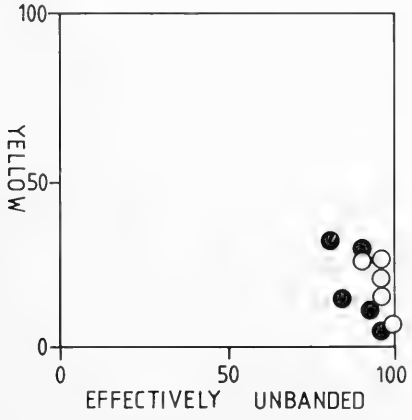

FIG. 8. Scatter of % yellow and % effectively unbanded in *C. nemoralis* in Whiteparish. Open circles, open habitats; solid circles, woods. All but one sample come from downland.

downland and ancient woods. Variation with habitat in downland populations is relatively weak, and chiefly involves fusions of bands.

By contrast, areas of more stable habitats show pronounced variation with habitat in fusions, colour and banding. Microgeographical variation is generally weaker, but present, and is particularly strong for unbanded in Whiteparish. Between sample correlations of various morphs, and the direction of variations with habitat both suggest the influence of visual selection for crypsis (Cain & Sheppard, 1954). Analysis of variance confirms the pattern described above, showing the greater contribution of geography in downland and of habitat in stable areas. Variation in fusions (Clarke, 1960, 1962) and in colour and banding (Carter, 1968) with habitat in *C. hortensis* are known from other areas, and likewise attributed to visual selection.

In *C. nemoralis* downland populations show no variation with habitat, and do show clear area effects for mid-banded. In stable areas, only woodland populations were found; these differ markedly from those from downland woods, and have very similar morph-frequencies to those reported from other stable areas where visual selection is effective (Cain & Sheppard, 1954; Currey, Arnold & Carter, 1964; Greenwood, 1974).

Variation with habitat may also be a consequence of climatic selection mediated by the reflectance and absorbance of heat by the shell (Jones *et al.,* 1977). With respect to colour, climatic and visual selection will tend to have similar consequences, but with respect to banding their effects will be different, with effectively unbandeds being favoured in woods under visual selection, and in the open under climatic selection. In this study, the evidence suggests that visual selection is the more important, since effectively unbandeds tend to be most frequent in woods. In areas

where non yellow *C. hortensis* is rare, and the principal variation between habitats is in fusions, the two hypotheses cannot be distinguished (Clarke, 1960).

Taken together, these results support the hypothesis that variation in downland populations of *Cepaea* is more subject to historical effects than that in populations in more stable areas. They are concordant with the conclusions of Cameron *et al.* (1980), and strengthen them not only by repetition, but by the more detailed correspondences made possible by considering individual site histories.

The idea that population history may influence present patterns of variation in *Cepaea* is not new. It has taken a variety of forms, and been used mainly, but not exclusively in the context of area effects. Goodhart (1963) suggested that past bottlenecks and isolation had led, via founder effect, to population groups with differing strongly co-adapted gene complexes, while Clarke (1966) and Johnson (1976) suggested that similar strong co-adaptations might develop parapatrically without previous isolation.

The role of accident in the form of genetic drift, once given primacy in explanations of *Cepaea* variation, is now minimized. The size of most area effects rules it out as a general explanation (Cain & Currey 1963a) and it is likely to be of importance only in exceptionally small and transient populations (Brussard, 1975; McCracken & Brussard, 1980).

Most other references to the effects of population history on variation in *Cepaea* take the mild and non-specific form of suggesting that the internal genetic balance of populations will influence their response to the external environment, which therefore does not completely specify the type of variation found (Cameron, Carter & Haynes, 1973; Jones & Irving, 1975; Harvey, 1976).

In some cases, the same visual response to the external environment may be achieved by a variety of genetic patterns. Hence, effectively unbanded and dark give clearer patterns of variation with habitat than do any of their constituent morphs (Cain & Sheppard, 1954; Clarke, 1962; Murray, 1966), although the authors concerned do not ascribe the underlying genetic variation to historical factors.

Such differences in the internal genetic milieu could clearly arise as a consequence of founder effect in the classic sense (Mayr, 1942, 1963), and the isolated and small populations of *Cepaea* on grazed downland present suitable opportunities for such effects. It is, however, not necessary to invoke random changes in such populations, since small and isolated populations may also acquire particular and perhaps unusual gene-frequencies as a consequence of strong local selection. If other genes are involved in any subsequent evolution of modifiers or co-adaptation, the necessary change in internal balance can arise as a consequence of selection, and transmit its effects to daughter populations in any subsequent expansions. The number of loci involved need not be great, and theoretical analyses (Slatkin, 1977; Wright, 1978) suggest that patterns of micro-geographical variation similar to area effects in *Cepaea* can arise in the absence of massive co-adaptation complexes of the sort that might lead to parapatric speciation.

In this context, it is unfortunate that the term 'area effect' is now used rather indiscriminately for a variety of patterns of local variation in many species, and has even led to the term 'area effect speciation' (White, 1978). In *Cepaea*, the evidence suggests that area effects are one or few locus phenomena, and that boundaries for effects at different loci do not generally coincide (Cain & Currey, 1963a; Harvey, 1976; Jones *et al.*, 1980). Even in other molluscs, superficially similar patterns of variation appear to have a variety of causes and details of genetic interactions. Woodruff & Gould (1980) report three separate instances of area effects in the Caribbean land snail *Cerion,* and ascribe them to parapatric divergence, isolation and subsequent hybridization, and to distant transport of a propagule respectively, the last resulting from an introduction made in 1912, the consequences of which persist to this day despite hybridization with local populations. Local variation in *Partula* on Moorea also seems to involve a variety of mechanisms, and to be rather unlike the situation in *Cepaea* (Clarke & Murray, 1969).

Even within *Cepaea*, it is not clear that area effects are a distinct kind of variation with a particular set of causes. Large scale geographical clines (Jones *et al.*, 1977), and the vaguer regional variation in colour polymorphism in *C. hortensis* (Carter, 1968) are excluded, but the range of patterns claimed as area effects is nevertheless considerable. In some downland areas, particular effects may occur over several km^2 (Cain & Currey,

1963a; Carter, 1968) but many much smaller patterns are also known—occupying only a few tens of meters in some linear populations (Goodhart, 1962 and 1973; Wolda, 1969), and on the one time downland of Silbury Hill, *C. hortensis* shows considerable variation within an area c. 300 × 200 m (Wall, Carter & Clarke, 1980). Downland area effects on Salisbury Plain and in this study are generally less clearly defined, and involve less extreme morph-frequencies than on the Marlborough Downs, and yet analysis shows pronounced microgeographical patterns on a smaller scale over distances from 50–500 m, rather similar to those reported by Goodhart (1973).

There is also a body of evidence to suggest that the strength and definition of area effects varies with the loci concerned in *C. nemoralis.* Area effects involving midbanded are especially clear, those involving banded/unbanded less so, and those for colour least of all (Cain & Currey, 1963a,b; Carter, 1968; Bantock & Noble, 1973; Jones & Irving, 1975; Jones *et al.,* 1980). Midbanded area effects in *C. nemoralis* are the most evident in this study.

In this study, areas of stable habitat show some geographical variation in addition to the more pervasive variation with habitat, especially at Whiteparish. The case is not unique, as there are other reported cases of area effects interacting with variation with habitat, both on downland, and in more stable habitats in both species of *Cepaea* (Cain & Currey, 1963a; Carter, 1968; Cameron, 1969 and in prep.). In these cases it is usually colour variation which shows the strongest response to habitat, and banding variation to geographical position. The detection of microgeographical variation in circumstances where other forms of variation are dominant can present problems (Jones *et al.,* 1980; Cameron, in prep.), but there seems little doubt that it will be found to be present in most circumstances. The distinguishing feature of downland *Cepaea* populations is not so much the presence of micro-geographical variation or area effects, although they are indeed present, as it is the absence, or weakness of response to other environmental factors, and especially to habitat. That such absence is associated with habitat and population instability and periodic isolation is entirely compatible with the hypothesis that population history has influenced variation in the ways described above. That differences in genetic background may persist even in more stable areas is suggested by the presence of microgeographical variations there; that such differences are less extreme, and do not inhibit other patterns of variations is to be expected given the greater degree of population continuity than on downland.

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LITERATURE CITED

ANDREWS, J. & DRURY, A., 1773, *Topographical map of Wiltshire* (facsimile edition 1952). *Wiltshire Records Society Publications,* vol. 8.

ARNOLD, R. W., 1970, A comparison of populations of the polymorphic land-snail *Cepaea nemoralis* (L.) living in a lowland district of France with those in a similar district in England. *Genetics,* 64: 589–604.

ARNOLD, R. W., 1971, *Cepaea nemoralis* on the East Sussex South Downs, and the nature of area effects. *Heredity,* 26: 277–298.

BANTOCK, C. R. & NOBLE, K., 1973, Variation with altitude and habitat in *Cepaea hortensis* (Müll.). *Zoological Journal of the Linnean Society of London,* 53: 237–252.

BRUSSARD, P. F., 1975, Geographic variation in North American colonies of *Cepaea nemoralis. Evolution,* 29: 402–410.

CAIN, A. J. & CURREY, J. D., 1963a, Area effects in *Cepaea. Philosophical Transactions of the Royal Society of London,* ser. B, 246: 1–81.

CAIN, A. J. & CURREY, J. D., 1963b, Area effects in *Cepaea* on the Larkhill Artillery Ranges, Salisbury Plain. *Journal of the Linnean Society of London, Zoology,* 45: 1–15.

CAIN, A. J. & CURREY, J. D., 1963c, The causes of area effects. *Heredity,* 18: 467–471.

CAIN, A. J. & SHEPPARD, P. M., 1954, Natural selection in *Cepaea. Genetics,* 39: 89–116.

CAMERON, R. A. D., 1969, The distribution and variation of three species of land snail near Rickmansworth, Hertfordshire. *Journal of the Linnean Society of London, Zoology,* 48: 83–111.

CAMERON, R. A. D., CARTER, M. A. & HAYNES, F. N., 1973, The variation of *Cepaea nemoralis* in three Pyrenean valleys. *Heredity,* 31: 43–74.

CAMERON, R. A. D., CARTER, M. A. & PALLES-CLARK, M. A., 1980, *Cepaea* on Salisbury Plain: patterns of variation, landscape history and habitat stability. *Biological Journal of the Linnean Society of London,* 14: 335–358.

CAMERON, R. A. D. & PANNETT, D. J., 1980,

Hedgerow shrubs and landscape history: some Shropshire examples. *Field Studies,* 5: 177–194.

CAMERON, R. A. D., WILLIAMSON, P. & MORGAN-HUWS, D. I., 1977, The habitats of the land-snail *Cepaea nemoralis* (L.) on downland and their ecogenetic significance. *Biological Journal of the Linnean Society of London,* 9: 231–241.

CARTER, M. A., 1968, Studies on *Cepaea II:* Area effects and visual selection in *Cepaea nemoralis* (L.) and *Cepaea hortensis. Philosophical Transactions of the Royal Society of London,* ser. B, 253: 397–446.

CLARKE, B. C., 1960, Divergent effects of natural selection on two closely related polymorphic snails. *Heredity,* 14: 423–443.

CLARKE, B. C., 1962, Natural selection in mixed populations of two polymorphic snails. *Heredity,* 17: 319–345.

CLARKE, B. C., 1966, The evolution of morph-ratio clines. *American Naturalist,* 100: 389–402.

CLARKE, B. C., ARTHUR, W., HORSLEY, D. T. & PARKIN, D. T., 1978, Genetic variation and natural selection in pulmonate molluscs. In FRETTER, V. & PEAKE, J., ed., *The Pulmonates,* vol. 2A, p. 219–270, Academic Press, London.

CLARKE, B. C. & MURRAY, J. J., 1969, Ecological genetics and speciation in land snails of the genus *Partula. Biological Journal of the Linnean Society of London,* 1: 31–42.

CUNLIFFE, B., 1973, Charlton, Hants.: the evolution of a landscape. *Antiquaries Journal,* 53: 173–190.

CURREY, J. D., ARNOLD, R. W. & CARTER, M. A., 1964, Further examples of variation of populations of *Cepaea nemoralis* with habitat. *Evolution,* 18: 111–117.

DARBY, H. C., 1977, *Domesday England.* University Press, Cambridge.

FINN, R. W., 1967, Wiltshire. In DARBY, H. C. & FINN, R. W., eds., *The Domesday Geography of south west England.* University Press, Cambridge.

FISHER, R. A., 1944, *Statistical methods for research workers,* ed. 9. Oliver & Boyd, Edinburgh.

GLOVER, J. E. B., MAWER, A. & STENTON, F. M., 1939, *The Place Names of Wiltshire.* University Press, Cambridge.

GOODHART, C. B., 1962, Variation in a colony of the snail *Cepaea nemoralis. Journal of Animal Ecology,* 31: 207–237.

GOODHART, C. B., 1963, Area effects and non-adaptive variation between populations of *Cepaea* (Mollusca). *Heredity,* 18: 459–471.

GOODHART, C. B., 1973, A sixteen-year survey of *Cepaea* on the hundred foot bank. *Malacologia,* 14: 327–331.

GOUGH, P. M., 1979, *Continuity and historical change in woodland and downland in an area west of Salisbury, Wiltshire.* Unpublished Ph.D. thesis, University of Birmingham.

GREENWOOD, J. J. D., 1974, Visual and other selection in *Cepaea:* a further example. *Heredity,* 33: 17–31.

GROSE, J. D., 1947, Botanical references in the Saxon Charters of Wiltshire. *Wiltshire Archaeological Magazine,* 51: 555–583.

GRUNDY, G. B., 1939, The ancient woodland of Wiltshire. *Wiltshire Archaeological Magazine,* 48: 530–598.

HARVEY, P. H., 1976, Factors influencing the shell pattern polymorphism of *Cepaea nemoralis* (L.) in East Yorkshire: A test case. *Heredity,* 36: 1–10.

HUDSON, W. H., 1900, *Nature in Downland.* Longmans Green, London.

JOHNSON, M. S., 1976, Allozymes and area effects in *Cepaea nemoralis* on the western Berkshire Downs. *Heredity,* 36: 105–121.

JONES, J. S. & IRVING, A. J., 1975, Gene frequencies, genetic background and environment in Pyrenean *Cepaea* populations. *Biological Journal of the Linnean Society of London,* 7: 262–271.

JONES, J. S., LEITH, B. H. & RAWLINGS, P., 1977, Polymorphism in *Cepaea:* A problem with too many solutions? *Annual Review of Ecology and Systematics,* 8: 109–143.

JONES, J. S., SELANDER, R. K. & SCHNELL, G. D., 1980, Patterns of morphological and molecular polymorphism in the land snail *Cepaea nemoralis. Biological Journal of the Linnean Society of London,* 14: 359–388.

LANE-POOLE, E. H., 1959, Cranborne Chase. In CRITTALL, E., ed., *A History of Wiltshire,* vol. 4. Victoria History of the Counties of England. University of London, London.

MAYR, E., 1942, *Systematics and the origin of species.* Columbia University Press, New York, xii + 334 p.

MAYR, E., 1963, *Animal species and evolution.* Harvard University Press, Cambridge, Mass., xiv + 797 p.

McCRACKEN, G. F. & BRUSSARD, P. F., 1980, The population biology of the white-lipped land snail *Triodopsis albolabris:* genetic variability. *Evolution,* 34: 92–104.

MORGAN, F. W., 1935, Woodland in Wiltshire at the time of the Domesday Book. *Wiltshire Archaeological Magazine,* 47: 25-33.

MURRAY, J. J., 1966, *Cepaea nemoralis* in the Isles of Scilly. *Proceedings of the Malacological Society of London,* 37: 167–181.

PETERKEN, G. F., 1974, A method for assessing woodland flora for conservation using indicator species. *Biological Conservation,* 6: 239–245.

POLLARD, E., HOOPER, M. D. & MOORE, N. W., 1974, *Hedges.* Collins, London.

RACKHAM, O., 1980, *Ancient woodland, its history, vegetation and uses in England.* Arnold, London.

SLATKIN, M., 1977, Gene flow and genetic drift in a species subject to frequent local extinctions. *Theoretical Population Biology,* 12: 253–262.

TAYLOR, C. C., 1964, The Saxon boundaries of Frustfield, *Wiltshire Archaeological Magazine,* 59: 110–115.

TAYLOR, C. C., 1967, Whiteparish. A study of the development of a forest edge parish. *Wiltshire Archaeological Magazine,* 62: 79–102.

THORLEY, A., 1981, Pollen analytical evidence relating to the vegetation history of the chalk. *Journal of Biogeography,* 8: 93–106.

TITTENSOR, R., 1980, A sideways look at nature conservation in Britain. *University College London Discussion Papers in Conservation,* 29.

WALL, S., CARTER, M. A. & CLARKE, B. C., 1980, Temporal changes of gene frequencies in *Cepaea hortensis. Biological Journal of the Linnean Society of London,* 14: 303–318.

WHITE, M. J. D., 1978, *Modes of speciation.* Freeman, San Francisco.

WOLDA, H., 1969, Fine distribution of morph-frequencies in the snail *Cepaea nemoralis* near Groningen. *Journal of Animal Ecology,* 38: 305–327.

WOODRUFF, D. S. & GOULD, S. J., 1980, Geographic differentiation and speciation in *Cerion*—a preliminary discussion of patterns and processes. *Biological Journal of the Linnean Society of London,* 14: 389–416.

WRIGHT, S., 1978, *Evolution and the genetics of populations,* vol. 4. *Variability within and among natural populations.* University of Chicago Press Chicago.

APPENDIX

Sample scores for *C. hortensis* and *C. nemoralis* from Cranborne and Whiteparish. Site locations and habitat details are given in the main text. Abbreviations: *C. hortensis*; U = unbanded, EU = other effectively unbandeds, 5 = banded shells with at least 1 upper band, F = banded shells with upper bands fused. *C. nemoralis*; U = unbanded, M = midbanded, MS = midbanded with spread band, 5S = five banded shells with spread bands, 5 = five banded shells, EU = all other effectively unbanded shells.

Minor and rare variants are not shown separately in the tables. In *C. hortensis* dark lipped shells are present in many samples (especially in pinks) and in *C. nemoralis* both orange-banded and hyalozonate shells are found in a few samples. Detailed scores are available from R. A. D. C.

C. hortensis from Cranborne

Sample number	YELLOWS				PINKS				BROWNS				Total
	U	EU	5	F	U	EU	5	F	U	EU	5	F	
1	4		4	8	26		3	1					46
2	6	1	14		3		1				2		27
3	14		13	5	2		4						38
4	9	3	3		3		3		3				24
6			17			3	5						25
7	7		63	4	22								96
8	4		31	4	2		1						42
9	1		32	1	3		5		1				43
10	2		19		1		3	1					26
12	18		7										25
13	13		9	1					2				25
14	3		13	8	6	10	13	4			12	11	80
15	6		14				3						23
16	3		13	3	11	3	6	1					40
17	2		16	2	2		2						24
18	1		3	3	4		6	5					22
19	4		12	3	8	4	10	4	16				61
20	5		10	1	2	1	3	1	5				28
21	5		14	5	5	4	7	2	1				43
22	6		9	1	3	2	4		2				27
23	6		4	12	1		5	5				3	36
24			6	4	1		4	3					18
25			12	6			44	5					67
27	5		17		1		1				5		29
28	7		24	1	1		2				2	1	38
29	4	1	13	1			1				2	2	24

C. hortensis from Cranborne (Continued)

Sample number	YELLOWS				PINKS				BROWNS				Total
	U	EU	5	F	U	EU	5	F	U	EU	5	F	
30	1		12		1		5				2		21
31	8		21	1		1					2		32
32	23		27		1	9	19						71
33	6	1	23	4	1	1	13	3					60
34	17		15	1	1	4	5						40
35	3		10	1		8	16						34
36	1		27	2	1	5	6						45
37	7		16		2	3	3				3		37
38			11	2		10	9						25
39			6	1		6	8						25
40	4		15	14	1		28	6					74
41	2		30	2									34
42	6		4	4	6	3	12	5					40
43	3		13	1	2		7	1					27
44	1		3	3	2		4	4					17
45	2	1	14	4	5	6	3				3	4	42
46	2		14	1	1		3						21
47	10		25	2	16		24						77
48	6		9	2	5	1	10						33
49	4		35	9			2		1		3		54
50	1		13	3	2	1	5	2					27
51	9		3	4			1		5		1	3	26
52	4		40	7	2	18	4						75
53			19	1	1		3						24
56	7		11	2	6		6	5					37
57	3		12	4	3		10	3					35
58	1		5	3	5	5	16	1	11				47
59	5		3		1				17				26
60	11		10		13		5		15		1		55

C. hortensis from Whiteparish

Sample number	YELLOWS				PINKS				BROWNS				Total
	U	EU	5	F	U	EU	5	F	U	EU	5	F	
1	3		12	5	1	2					1	2	26
2	8		14	3									25
3			30	2			2						34
4	4	1	14	25	2	9	9	1					65
6			23	11	2		2		1		5	4	48
7	2		8	4	2							5	21
8			6	12	6	1	1	1	1				28
9	1		4	3	9		2	1	3				23
10	8		20	21	4		3	2	2				60
11	1		19	1	3								24
12	1	2	20	2			1						26
14	2		14	1	3								20
15	10		7	9	11		2		2			4	44
16	8		29	4	15		5				2	2	65
17			3	7	4				4		2	7	27
18	1		13	7		1		2				1	25
19			3	6	5		1	1					16

C. hortensis from Whiteparish (Continued)

Sample number	YELLOWS				PINKS				BROWNS				Total
	U	EU	5	F	U	EU	5	F	U	EU	5	F	
20	1		2	2	7		1		2		2	1	18
21			10	5			11		1			3	30
22			17		1		3						21
23	15		32	16	16	2			3		4	3	91
24	15		20	8	3	6	1				2	4	59
25	3	1	3	5	24				3			3	42
26	19		11	7	2							1	40
27			7	8	1						1	3	20
28	2		4	2	13		1		3		2	4	31
29	1		20	8	4						3	2	38
30	6		7	4	9	5	7		2		5	6	51
31	2	1	5	3	8	3	1		5		2	4	34
32	2		5	1	9		1		3				21
33	4		10	4	8						4		30
34	7	1	32	12	8	1	12	4			2	3	82
35	7		9	1	5	1							21
36	13		10	5	1				2		2		33
37	5		12	2	14				5		1	4	43
38	5		9	4	9				2		3	2	34
39	2		13	4	6		1				1	2	29
40	1		6	1	2		8	3					21

C. nemoralis from Cranborne

Sample number	YELLOWS						PINKS						BROWNS						Total
	U	M	MS	5S	5	EU	U	M	MS	5S	5	EU	U	M	MS	5S	5	EU	
1							2	7	1	2	5		2						19
3	2				9	1	2				5		2						21
4	1				14						19								34
5	3				11		2	1			4								21
6	3	14			4		98	2		1	7	1	5						135
11				9	26	2	1			2	9		9						60
12				11	26	1	2			1	4	1	4						54
13				5	20		1	1		1	13		1						42
14							11	6	8	4	3								32
18		2			2	1	4	2	2		1		8						22
19					2		1	2			7	2							14
26	1			17	21	9	8			3	10	6	4						79
29					6	3					16	3							28
30					2	1					4	2							9
38				3						3	6		1						13
39		1						6	3		2		2						14
40		1			2		2	11	9		5		2				1		33
45							3	4			5	2	1						11
48		1			3			3		5	18		3						20
50		1			1			1	2	1	12								12
51							1	4		10	3								33
54	3	18	6	1	14		1	12	3	1	4	1	10	1			1		84
55	1	4		1	7	1	3	1					1						22
58		1			1	2	2	3					1						13

C. nemoralis from Whiteparish

Sample number	YELLOWS						PINKS						BROWNS						Total
	U	M	MS	5S	5	EU	U	M	MS	5S	5	EU	U	M	MS	5S	5	EU	
1	4	8					11	47			5		6	2			1		84
2		3						6			1								10
4	3	8			5			12					24	1					53
5		6			1		9	7					5						28
9		2			3		1	3	3				2	1					15
13		4			1		2	14					1					1	23
17	1	1					3	28	3		1			1					38
18		1						7	2				2	5					17
23	1	4					2	20			1		4	5					37
24		6					2	31			3		7	2					51

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EXPERIMENTAL STUDIES ON THE EFFECTS OF DENSITY, SIZE, AND SHELL COLOUR AND BANDING PHENOTYPES ON THE FECUNDITY OF *CEPAEA NEMORALIS*

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ABSTRACT

Snails of different size and phenotype were collected from natural populations at different densities. They were maintained at three different experimental densities during the egg laying season. The effects on various fecundity components of differences in population of origin, size of snail, snail phenotype and experimental density were measured. There was a consistent reduction in fecundity as a result of crowding in the parent population or during the experiment. Fecundity was positively correlated with size within but not between populations of origin. Differences in shell phenotype had little or no effect on fecundity. The results of the experiments are discussed in relation to a density dependent model of population regulation in *Cepaea nemoralis*.

Key words: *Cepaea*; snail; size; shell phenotype; density; fecundity; population regulation.

INTRODUCTION

There has been recent interest in the mechanisms which regulate the population sizes of gastropod molluscs. The growth rate and fecundity of several fresh water and terrestrial snails are influenced by population density (Berrie & Visser, 1963; Butler, 1976; Eisenberg, 1966, 1970; Mooij-Vogelaar, Jager & Van der Steen, 1970, 1973; Thomas & Benjamin, 1974; Pomeroy, 1969; Wright, 1960; Yom-Tov, 1972). Most studies have demonstrated an inverse relation between density and measured variables but Thomas & Benjamin (1974) found growth and fecundity maxima at an intermediate density. Food quantity or food quality have been considered to be the limiting resource in many of these studies but Thomas, Goldsworthy & Benjamin (1974) found that the density effects in the freshwater *Biomphalaria glabrata* were due to chemical conditioning of the medium by the snails.

Both natural and artificial populations of the land snail *Cepaea nemoralis* (L.) show strong negative relationships between adult shell size and snail density (Williamson, Cameron & Carter, 1976; Cook & Cain, 1980; Carter, Ashdown & Morgan-Huws, in preparation). In the laboratory the activity and growth of juvenile *C. nemoralis* are significantly reduced at increased densities probably because of an increased deposit of mucus (Oosterhoff, 1977; Cameron & Carter, 1979).

Wolda (1963, 1967) and Wolda & Kreulen (1973) showed a positive correlation between the size of parent snails and the number and size of egg clutches laid. If there is a strong environmental component to size and if larger *C. nemoralis* adults are more fecund than small ones then the effect of density on the size of adult snails could be important in the regulation of numbers of this species.

This paper describes the results of experiments designed to investigate the effects of size and density differences on the fecundity of *C. nemoralis* individuals taken from two neighbouring populations in southern England. These populations have different mean shell sizes related to their different field densities, and like most other populations they are polymorphic for shell colour and band pattern. We have therefore also investigated the fecundities of the most frequently-occurring phenotypes.

MATERIALS AND METHODS

Three experiments were carried out between June and September in 1978, 1981 and 1982. They all followed a similar pattern. In the first year, size and density effects were investigated. The second and third year's ex-

periments were concerned with the fecundities of various shell colour and shell band pattern phenotypes at different densities.

Adults for all three experiments were collected in May, each time within an area of 400 m^2. This is smaller than the panmictic area for this species (Murray, 1964). In 1978 snails were collected from two populations A and B, of high and low field density respectively, which were 1 km apart on chalk grassland in a dry valley on the South Downs (National Grid SU 804187 and SU 802177). The populations contained snails of different mean size but overlapping range. The snails for the 1981 experiment were collected from population A in which pink and yellow shells were approximately equally frequent and over 90% of shells were five-banded. By 1982, population B containing mid-banded as well as five-banded individuals was very sparse, so these phenotypes, whose fecundities were to be tested in the third experiment, had to be collected from population C on a chalk grassland site 80 km away on the Lambourn Downs (National Grid SU 300800).

For the first experiment, the maximum diameter of the shells was measured from the lip across the umbilicus. Snails were classified into one of three size classes, small (18.5–19.9 mm), medium (20.0–21.4 mm) and large (21.5–23 mm). Snails from population A were medium or small, those from population B were medium or large. Most snails had relatively undamaged periostraca and probably had been adult for less than three or four years (Williamson, 1979). Some snails from population A had very worn periostraca and they were likely to have been adult for at least five years. Five categories of snail were defined in this experiment. The three from population A were young, medium size (mean diameter 20.6 mm); young, small size (mean diameter 19.5 mm); and old, small size (mean diameter 19.4 mm). The two categories from population B were young, medium size (mean diameter 20.7 mm); and young, large size (mean diameter 22.1 mm). There is no correlation between shell phenotype and shell size in these populations (Carter, Ashdown & Morgan-Huws, in preparation) so that different phenotypes were distributed at random among the size categories.

For the second experiment shells were scored for pink or yellow colour. All shells were five banded. Three categories were defined in this experiment: all shells pink; all shells yellow; and equal numbers of pink and yellow shells. In the third experiment shells were scored for band patterns and categorised as mid-banded or five-banded. All shells were yellow. There were no significant size differences between the shell colour or banding categories in experiments two and three.

In all experiments individuals within each category were assigned to one of three densities at random. These densities were:—two snails per container, four snails per container or eight snails per container. In the first experiment each size class was replicated six times per density and in the other two experiments there were ten replicates of each colour or banding category per density.

The containers were 0.25 m diameter flowerpots part filled with limed soil to uniform specification and covered with a sheet of glass. The soil was 0.15 m deep with a surface area of 0.03 m^2. The total surface available to snails within a container was 0.15 m^2. Each snail was fed weekly on a slice of carrot 1.0 cm thick and 3.0 cm diameter, so that there were 2, 4 or 8 slices per container. At all densities, there was some carrot left at the end of each week; this was removed. There were several lumps of quarry chalk in each container. The containers were randomised in partial shade. They were inspected weekly, at which time the soil was watered if necessary.

C. nemoralis, like many other terrestrial gastropods, buries its egg clutches. During the period June to September the soil in the containers was lightly turned over at weekly intervals. In this way every clutch was found, then removed whole to a standard plastic petri dish lined with moistened filter paper. The dishes were maintained in a shaded laboratory in the same array as the parent containers. Temperature was not controlled and maximum temperatures in the laboratory varied regularly above 20°C. This is the temperature above which Wolda (1963) found that hatching success was less than 100%. The newly laid clutches were weighed and the eggs were counted. One week after a clutch had started to hatch the number of juveniles was counted.

Very few snails were lost from the experiments. Those that were lost in the first two weeks were replaced by spare snails of the same category which had been kept under the same density conditions.

At the end of experiment 1 the snails were collected and killed by freezing. The bodies were removed from the shells and brought to

TABLE 1. Mean values of various fecundity components of five size and age categories from two populations of *Cepaea nemoralis*. Values of eggs per snail, clutches per snail and young per snail were calculated by dividing appropriate container totals by the numbers of snails in that container; values were then averaged over each category. Percentages in brackets in last column of table are the mean hatching success of the clutches in each category. Values for mean squares, variance ratios and probabilities of each analysis of variance are given under their respective fecundity component column headings.

Category	eggs/snail	clutches/snail	clutch size	egg wt. (gms $\times 10^{-3}$)	young/snail
Pop. A, small, old	116.91	3.4	35.62	11.00	66.59 (51%)
Pop. A, small, young	102.82	2.4	43.78	11.89	61.82 (56%)
Pop. A, medium, young	118.89	2.8	43.64	12.06	70.09 (54%)
Pop. B, medium, young	166.49	3.5	47.54	11.28	105.67 (60%)
Pop. B, large, young	195.60	3.5	56.61	12.44	133.76 (65%)
Analysis of variance (mean squares)					
Between categories 4 degrees of freedom	27694.44	4.60	1042.60	6.21	17414.06
Residual 75 degrees of freedom	481.64	0.38	32.54	1.16	342.28
Variance ratio Categories:residual	57.50	12.22	32.04	5.33	50.88
Probabilities	<0.001	<0.001	<0.001	<0.01	<0.001

a constant dry weight in a vacuum oven. Shells and bodies were then weighed separately.

RESULTS

Experiment 1: the effect of population of origin, size and density on fecundity

Experiment one was set up on 22nd May 1978.

The first clutches laid were found on 1st June. The majority of clutches were laid between that date and the middle of August. Only three clutches were laid between 31st August and 14th September when the experiment was ended.

The effects of population of origin, snail size and age on various fecundity components are shown in Table 1. Individuals from population A laid on average many fewer eggs (112.90) than individuals from population B (181). There are six comparisons possible between population A snails and population B snails. All are significant with $P < 0.001$ in each case. Of particular interest is the significant difference between snails of similar age and size but from different populations.

Smaller snails laid, on average, significantly fewer eggs than larger snails of the same age and from within the same population. Both possible comparisons are significant. The single within-population comparison for the effect of age on egg laying is not significant.

The differences between populations A and B in numbers of eggs per snail have two components. First, there are interpopulation differences in the average number of clutches laid (Table 1). There are four possible comparisons for snails of similar age and the differences in average number of clutches per snail are significant in each case. Second, there are interpopulation differences in average clutch size with five of the six possible comparisons being significant (Table 1).

In this experiment older snails lay smaller clutches more frequently than younger snails from the same population. The appropriate comparisons are all significant (Table 1).

Egg weight seems to be most affected by the size of the parent. The largest parents within both populations lay the heaviest eggs although only significantly so in population B. There is also an age effect. The older snails lay significantly lighter eggs than either of the groups of younger snails from the same population.

The final column in Table 1 shows the

TABLE 2. Mean values for various fecundity components of snails maintained at different experimental densities. Values were calculated as in Table 1. Final column percentages in brackets are average hatching successes at the different densities. Probabilities attached to variance ratios are given as **, $P < 0.01$, ***, $P < 0.001$.

Density	eggs/snail	clutches/snail	clutch size	egg wt. (gms $\times 10^{-3}$)	young/snail
2 snails/pot	154.32	3.45	45.64	11.53	102.85 (61%)
4 snails/pot	135.18	3.03	44.99	11.80	84.83 (56%)
8 snails/pot	130.92	2.87	45.69	11.87	75.08 (54%)
Analysis of variance (mean squares)					
Between densities 2 degrees of freedom	4657.40	2.66	4.64	0.93	5955.88
Snail category—density interaction 8 degrees of freedom	974.72	0.63	47.72	0.20	435.43
Residual 75 degrees of freedom	481.64	0.38	32.45	1.16	342.28
Variance ratio densities:residual	9.67***	7.07**	0.14	0.80	17.40***

average number of newly hatched juveniles produced by the various categories of parents. The major difference is between the two populations. Each of the six possible comparisons is significant with $P < 0.001$. There is also a size effect, although this is only significant within population B. The proportion of eggs which hatches is not constant for the various parental categories. The figures in brackets beside the last column in Table 1 show that on average a higher proportion of live young emerges from clutches laid by population B individuals. Analysis of variance of appropriately transformed data shows that these differences are significant.

The effects of laboratory density on fecundity during this experiment are shown in Table 2. There is a significant decrease in the number of eggs laid with the increase in density. This effect is entirely due to a significant decrease in the number of clutches laid, as there is no effect of clutch size (Table 2). Egg weight is not affected by density. The number of live young produced by the snails depended on the number of eggs laid and the hatching success of those eggs. The figures in brackets in the last column of Table 2 show that the eggs of parents at different densities had different average hatching success. These differences are significant with $P < 0.05$.

Experiment 2: the effect of shell colour and density on fecundity

This experiment was set up on 28th May 1981 and the first clutches were laid on 12th June. Eggs were laid over a shorter period than in the first experiment. Table 3 shows the effect of snail shell colour and snail density on various fecundity components. Pots which contained only pink five-banded shells produced the greatest average number of eggs per snail. This effect is not significant in the three way comparison but it is significant in a two way comparison between pure pink and pure yellow snail containers. The number of eggs laid in the mixed pots is almost exactly intermediate between those in the single-colour pots. The number of eggs laid was determined by the number of clutches produced; there are no significant differences in clutch size. There are no significant differences in the average number of young produced by the three snail colour categories; the differences in numbers of eggs laid was counterbalanced by a significant difference in the hatching success of those eggs.

The effects of density on fecundity exactly parallel those found in experiment 1. There is a significant decrease in the number of eggs laid at increased densities. This is related to a significant decrease in the number of clutches

TABLE 3. Mean values for various fecundity components of two shell colour phenotypes maintained in pure and mixed cultures at three different densities. Values were calculated as in previous tables. Appropriate mean squares, variance ratios and their significance are given. Probabilities attached to the variance ratios are given as *, $P < 0.05$, **, $P < 0.01$. Means and standard deviations of shell sizes were: pink 20.0 ± 0.08 mm., yellow 19.9 ± 0.8 mm.

Category	eggs/snail	clutches/snail	clutch size	egg wt. (gms × 10^{-3})	young/snail
Pink	73.70	2.05	36.51	12.08	36.24 (36%)
Pink + Yellow	66.38	1.75	39.25	12.16	32.46 (41%)
Yellow	58.81	1.56	39.40	12.37	36.05 (49%)
Density					
2 snails/pot	76.78	2.03	38.28	12.69	43.90 (43%)
4 snails/pot	66.09	1.90	36.04	11.96	34.12 (43%)
8 snails/pot	56.01	1.43	40.84	11.97	24.54 (39%)
Mean squares					
Colour 2 d.f.	1663.43	2.17	79.08	5.24	136.28
Density 2.d.f.	3237.11	2.79	172.95	0.68	646.28
Interaction 4.d.f.	658.00	0.25	39.75	3.97	113.78
Residual 81 d.f.	578.43	0.62	62.99	2.32	187.38
Variance ratios					
Colour:residual	2.88	3.88*	1.26	2.52	0.73
Density:residual	5.60*	6.49**	2.75	0.29	3.45*
Interaction:residual	1.14	0.39	0.55	1.71	0.61

laid; the differences in clutch sizes are not significant. The number of live young produced immediately post-hatching depended on the number of eggs produced, in this experiment there was no significant effect of density on hatching success of the eggs.

There is no evidence that the snails with different shell colours respond differently to increased density; none of the interaction variance ratios is significant.

Experiment 3: the effect of shell band pattern and density on fecundity

This experiment commenced on 30th May 1982. The eggs were laid over a similar period to experiment 2. Table 4 shows that there were no significant effects of shell band pattern on fecundity. The mid-banded and five-banded snails produced very similar numbers of young. The effects of density are very similar to those in the other two experiments. There are significant reductions in the average number of eggs per snail and the average number of newly hatched young produced by each snail as density increases. As before, the decrease in number of eggs is due to a significant reduction in the number of clutches laid. The number of newly hatched young produced at the different densities depends in the main on the number of eggs laid; the hatching success of those eggs is not significantly affected by density. The two types of banded snail do not respond differentially to density; there are no significant interaction variance ratios.

Changes in fecundity with time

The data for each experiment were partitioned to investigate any changes in fecundity during the egg-laying season. In all the experiments the mean sizes of the clutches laid during the first half of the season (up to 16th July) are significantly greater than those of the later clutches (Table 5).

There are differences between experiments but overall there is a trend for a gradual decrease in clutch size with time. In all the experiments significantly more clutches were laid in the first half of the season but only in experiment 3 is there a steady decrease in the number of clutches with time (Table 5).

The data were analysed for any season-related differential effects of density on the numbers of eggs and clutches laid as well as the size of the clutches. There were no consistent differences.

TABLE 4. Mean values for various fecundity components of two shell banding phenotypes maintained in pure cultures at three different densities. Values were calculated as in Tables 1 and 2. Appropriate mean squares and variance ratios and their significance are given. Means and standard deviations of shell sizes were: mid-banded 20.8 ± 1.0 mm, five-banded 20.5 ± 1.1 mm.

Category	eggs/snail	clutches/snail	clutch size	egg wt. (gms × 10^{-3})	young/snail
Mid-banded	69.52	1.84	37.20	13.10	32.97 (46%)
Five-banded	62.66	1.72	37.66	12.97	30.83 (49%)
Density					
2 snails/pot	84.28	2.33	36.83	13.54	41.95 (55%)
4 snails/pot	63.99	1.71	36.84	13.04	29.82 (44%)
8 snails/pot	50.00	1.31	38.62	12.54	23.91 (43%)
Mean squares					
Banding 1 d.f.	706.30	0.25	3.21	0.22	68.80
Density 2. d.f.	5941.15	5.24	21.31	4.98	1690.58
Interaction 2 d.f.	470.41	0.48	54.00	1.89	222.57
Residual 54 d.f.	1167.38	0.67	62.50	4.31	258.22
Variance ratios					
Banding:residual	0.61	0.37	0.05	0.05	0.27
Density:residual	5.08*	7.78**	0.34	1.16	6.55**
Interaction:residual	0.40	0.71	0.86	0.44	0.86

TABLE 5. Number and mean size of clutches produced by snails from three populations at six fortnightly intervals during the laying season. Experiment 1 was carried out in 1978, experiment two in 1981 and experiment 3 in 1982.

End date for sampling interval	Experiment 1				Experiment 2		Experiment 3	
	Pop. A (252 snails)		Pop. B (168 snails)		Pop. A (420 snails)		Pop. C (280 snails)	
	No.	size	No.	size	No.	size	No.	size
16 June	75	57.05 ± 2.47	126	84.52 ± 2.52	—	—	—	—
31 June	182	56.93 ± 2.25	135	58.34 ± 2.03	181	40.39 ± 1.38	210	41.81 ± 0.97
16 July	137	35.33 ± 1.31	93	45.24 ± 1.05	306	40.75 ± 1.11	161	35.16 ± 1.19
31 July	168	30.18 ± 0.93	126	35.60 ± 1.15	140	31.84 ± 1.01	66	30.15 ± 1.27
16 Aug	98	23.03 ± 0.90	70	28.79 ± 1.48	65	19.32 ± 1.35	2	30.50 ± 3.50
31 Aug	20	21.60 ± 2.88	13	21.31 ± 2.47	—	—	—	—

Differences in snail behavior

When the containers were examined during September 1978 it was noted that some snails were aestivating and had laid down a white calcareous epiphragm. When the experiment was ended on 14th September the numbers of active and aestivating snails in each container were noted. Table 6 shows the effect of snail category and density on snail activity at this time. There is a significant increase in the proportion of aestivating snails among those which had been at higher densities (Friedman's statistic $\chi^2_r = 6.4$ $P < 0.05$). The difference between snail categories is not significant.

Body weights and shell weights

The average dry body weights and shell weights of the five snail categories at the end of experiment 1 are shown in Table 7 together with the average weights of animals that had been at the three different densities. It was not possible to estimate body or shell weights before the experiment but once each snail had been categorised the members of each category were distributed among the densi-

TABLE 6. The proportions of snails of different age, size and origin which were found aestivating on 14th September 1978 at the end of an experiment in which they had been maintained at three different densities.

Snail category	Crowding, snail per container		
	2	4	8
Pop. A, small young	0	8%	2%
Pop. A, small old	0	0	3%
Pop. A, medium young	0	0	8%
Pop. B, medium young	0	8%	15%
Pop. B, large young	8%	18%	23%

TABLE 7. Mean values of dry body weight and shell weight of snails of different origin and various size and age categories. Snails had been maintained at various experimental densities during the summer of 1978. Measurements were made at the end of the experiment. Body weight was brought to a constant value in a vacuum oven maintained at 60°C. Significance levels of analysis of variance are * $P < 0.05$, *** $P < 0.001$.

Snail category	dry body wt. (gms)	shell wt. (gms)
Pop. A small old	0.184	0.607
Pop. A small young	0.218	0.734
Pop. A medium young	0.238	0.800
Pop. B medium young	0.233	0.840
Pop. B large young	0.289	0.942
Density		
2 snails/pot	0.233	0.824
4 snails/pot	0.231	0.780
8 snails/pot	0.232	0.750
Mean squares		
Snail Category d.f. 4	0.0265	0.281
Density d.f. 2	6.8×10^{-5}	0.041
Interaction d.f. 8	4.2×10^{-4}	4.85×10^{-3}
Residual d.f. 75	5.0×10^{-4}	0.011
Variance Ratios		
Snail category:residual	53.42***	26.23***
Density:residual	0.14	3.84*
Interaction:residual	0.84	0.45

ties using random number tables. It is therefore unlikely that there were initial systematic differences between the snails at the various densities. There were no density-related differences in body weight at the end of the experiment but there was a systematic reduction in shell weight at increased densities which was significant.

The differences between pairs of snail categories except those between snails of the same size were significant for body weight and shell weight. As might be expected larger animals had heavier bodies and shells. Older animals had lighter bodies and shells than younger ones of the same size. Snails from different populations but of the same size had very similar shell and body weights.

DISCUSSION

The effects of field and laboratory density on fecundity

The main feature of this study has been the consistent reduction in fecundity as a result of crowding snails, either in the laboratory at the time of laying or previously in the field. The densities in the natural populations from which snails were obtained for the first experiment were estimated as 0.5 (population B) and 5.0 adults m^{-2} (population A). During the experiment the fecundities of the snails from the two populations were significantly different (Table 1). The age and size components of these differences are discussed below, but a most important result is that snails from the less dense population B were significantly more fecund than snails of similar age and size from the more dense population A. The field densities seem to have had their residual effect on snail fecundities mainly during the first two weeks of the experiment (Fig. 1). After that time differences between snails from the two populations were much less extreme.

Experimental densities were relatively high, varying from 13 to 53 adults m^{-2}. This compares with the densities in the parent populations (0.5 and 5.0 adults m^{-2}) and maximum field densities at other sites of 12 to 15 adults m^{-2} (Cain & Currey, 1968; Price & Bantock, 1975). Nevertheless the values of the various fecundity components that we obtained were mostly within the upper range of estimates obtained by Wolda and his coworkers for a series of natural and artificial populations and were similar to the values they obtained in a study of a semi-natural population with 3.5 adults m^{-2} (Wolda, 1963, 1965, 1967; Wolda & Kreulen, 1973).

The number of eggs produced by the snails varied with density because fewer clutches

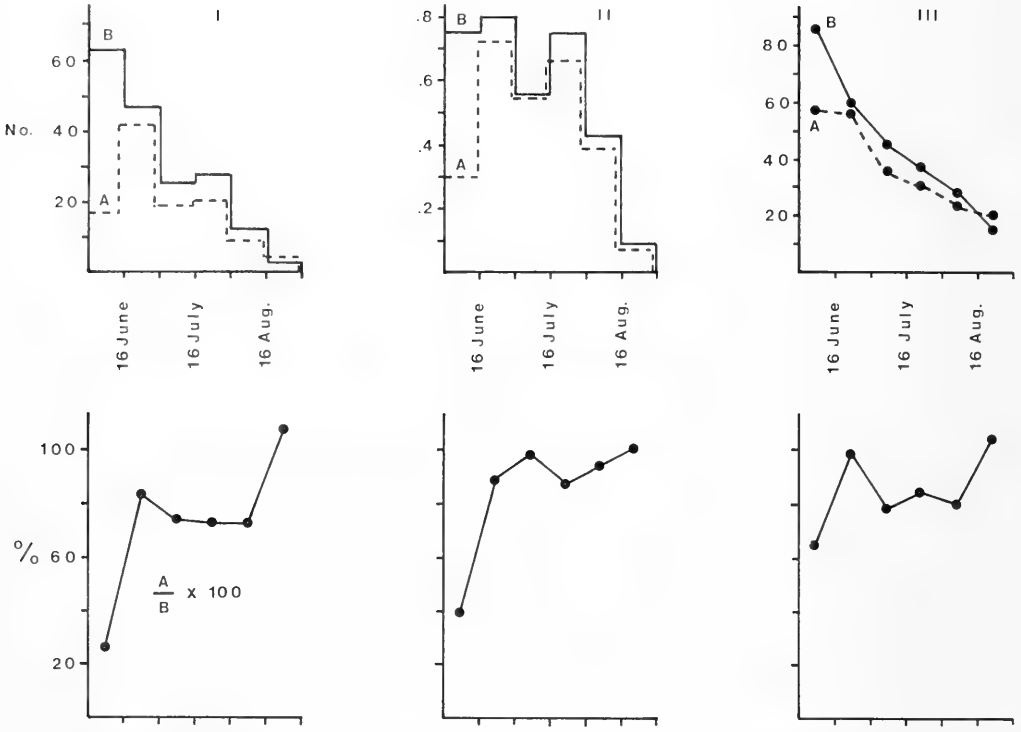

FIG. 1. The effect of high field density (population A) and low field density (population B) on three fecundity components averaged over two week intervals during experiment 1 (June to September 1978). I, no. of eggs, snail $^{-1}$, fortnight $^{-1}$. II, no. of clutches, snail $^{-1}$, fortnight $^{-1}$. III, mean clutch size, fortnight $^{-1}$. Ratios of values for snails from A and B are also given for ease of comparison.

were laid at the higher densities. There was no variation in clutch size. It was not possible to determine if snails responded uniformly or differentially to crowding because the experimental design meant that clutches could not be assigned to individual snails. Wolda (1963) investigated egg-laying of individual *C. nemoralis* over successive seasons. Clutch production did vary between snails but Wolda concluded that very little of this variation was genetically determined. There is therefore little reason to suppose that the response to density has a large genetic component. Other snail species produce fewer eggs as density increases but not all responses are identical. Under crowded conditions a smaller proportion of *Trochoidea seetzeni* lay, and they lay smaller clutches (Yom-Tov, 1972); *Bulinus forskalii* lays fewer and smaller clutches (Wright, 1960). *Lymnaea stagnalis* appears more similar to *C. nemoralis,* with a lower laying frequency but unchanged or larger clutch size (Mooij-Vogelaar, Jager & Van der Steen, 1970, 1973).

Various studies indicate possible mechanisms whereby fecundity might be inhibited at increased density. Vianey-Liaud (1979) showed that starved *Biomphalaria glabrata* steadily reduced the number of egg masses produced but did not alter the average number of eggs per egg mass. Pomeroy (1969) suggested as a result of his experiments that shortage of food might cause *Helicella virgata* at higher densities to produce fewer young. Eisenberg (1970) found that the reduction in the size of egg masses of *Lymnaea elodes* at higher densities could be prevented by addition of small quantities of spinach to experimental cages and he suggested that food quality might be limiting. Similar conclusions were reached by Mooij-Vogelaar & Van der Steen (1973).

In the experiments reported here, food was provided, in the form of carrot slices, in pro-

portion to snail density. Carrot was chosen because it is known to be assimilated with higher efficiency than several potential natural foods (Williamson & Cameron, 1976). The growth rate of carrot-fed laboratory snails is of the same order as snails in natural populations (Oosterhoff, 1977; Cameron & Carter, 1979). The similarity of dry body weights of snails from different densities at the end of experiment 1 suggests that the snails at the higher densities were not grossly starved. Although it is not possible absolutely to rule out food as a limiting resource in the present experiments, it seems unlikely that it was.

The lower shell weights found among snails which had been at higher densities might suggest that calcium was limiting in spite of the chalk which was always available. If so, lack of this resource did not appear to affect egg quality consistently since egg weights at the various densities within each experiment were similar and differences in hatching success were not always significant.

Another possibility is that fewer clutches were laid at higher densities because of competition for egg laying sites. There was a sufficient area of soil even at high densities for eight snails to bury their clutches simultaneously but they may have been in competition for preferred sites so that a burying activity of some snails may have interfered with this activity in others. However, clutches were never found on the soil surface.

The exchange of information between snails could result in the regulation of fecundity even in the absence of limiting resources. Cameron & Carter (1979) have shown that in *C. nemoralis* in the laboratory, adult activity is suppressed in the presence of exogenous slime. Oosterhoff (1977) showed that juvenile growth was suppressed in the presence of extra slime. It did not matter if this slime was wiped over both the food and the filter paper lining the container or only the latter. The snails responded in the same way to either treatment and were significantly slower growing than the controls. Cameron & Carter (1979) showed that snail activity was significantly reduced at increased density in the presence of excess food and we have shown here that adult aestivation is increased at high density irrespective of food. Juvenile *Helix aspersa* is less active in the presence of slime (Dan & Bailey, 1982) and this species also grows more slowly, is less active and less fecund and suffers higher mortality under crowded conditions (Herzberg, 1965; Dan & Bailey, 1982). Thomas, Goldsworthy & Arram (1975) isolated several growth-promoting substances from media conditioned by sexual *Biomphalaria glabrata.* They considered that some of these substances may have originated from the snails. Berrie & Visser (1963) obtained growth inhibiting substances from *B. glabrata* faeces although these may have been derived from the plant food.

It is not yet known if the *C. nemoralis* at high densities failed to develop mature gametes or broke them down. Starvation is known to cause gamete resorption in *Biomphalaria glabrata* (Vianey-Liaud, 1979). However it has also been shown that tentacle extracts injected into the body cavities of *Arion ater* and *Arion subfuscus* inhibit oocyte development (Pelluet & Lane, 1961). Slime, if it is the cause of density effects in *Cepaea nemoralis,* might act indirectly by reducing feeding activity or more directly by stimulating tentacular neurosecretions.

The effects of age, shell phenotype and shell size on fecundity

The results of the experiments reported here are in general agreement with those of Wolda and his colleagues (Wolda, 1963, 1965, 1967; Wolda & Kreulen, 1973; Oosterhoff, 1977). There are some differences, and in general our data suggest that it is important to take account of population of origin of the *C. nemoralis* used in fecundity studies, particularly when considering the effects and implications of size.

The effects of age were found to be exactly the same as in previous studies. Young snails laid fewer and larger clutches than older individuals of the same size and from the same population.

Differences in shell colour and banding phenotype appear to have only a small effect on fecundity. There were some differences in egg production between the colour phenotypes but none between the banding ones. There was no evidence of altered egg production by a phenotype in a monomorphic mating group as compared with a polymorphic one. Wolda (1963, 1965, 1967) found differences in both the number of clutches laid by different colour and banding phenotypes and the size of those clutches. Some of the differences were significant, some were not. In general the shell-phenotype-related fecundity differences he observed appear less clear-cut than

those relating to shell size. The fecundities of pink and yellow phenotypes responded to changes in density in the same way as far as we could detect and decreased as density increased. This was also true of the mid-banded and the five-banded phenotypes. We have evidence that different phenotypes have the same size distributions within natural populations (as they have within our experiments) and we know that shell colour and banding phenotype frequencies remain relatively stable in spite of density changes within those populations (Carter, Ashdown & Morgan-Huws, in preparation). It therefore seems unlikely that density has a major role in determining shell phenotype frequencies in natural populations.

As in previous experiments, snail size had a dramatic effect on fecundity. Within both the populations used, larger snails produced more eggs than smaller individuals of similar age. However, this was achieved in different ways, population A showing an increase in clutch number and population B an increase in clutch size. Thus within a population it is not always true as Wolda & Kreulen (1973) and Oosterhoff (1977) suggest that larger snails always produce more and larger clutches; indeed Wolda (1963, 1967) had previously found no correlation between these two fecundity components.

There is no absolute relationship between shell size and egg production. Snails of the same size and the same body and shell weights but from different populations can lay different numbers of different-sized clutches when placed under the same conditions (experiment 1). Further, snails of the same size range and from the same population can lay very different numbers of eggs in different years (experiments 1 and 2). Similar data were obtained by the Dutch workers.

As expected from Wolda's (1963) experiments, only a proportion of the eggs in each clutch hatched under our laboratory conditions, as temperatures varied above 20°C. There were significant differences in hatching success related to population of origin (experiment 1) and shell phenotype (experiment 2). There was no effect of parent size within population in our data. This contrasts with the conclusions of Oosterhoff (1977). It is not possible to determine whether size and populations of origin are confounded in her data, but it does not seem to be generally true that hatching success is directly related to size.

The changing pattern of egg laying during the season that occurred in all our experiments seems to be a universal feature of the reproductive behaviour of *Cepaea nemoralis* (Wolda, 1963; Wolda & Kreulen, 1973; Oosterhoff, 1977). We agree with Oosterhoff that the decrease in clutch size is unlikely to be due to a change in the quality of food, as the same pattern that occurs in natural populations also occurs in experiments where food quality and quantity are kept constant.

The data presented here are consistent with a density-dependent model of population regulation in *Cepaea nemoralis.* In natural populations, snail size is much reduced at higher densities (Williamson, Cameron & Carter, 1976) and juvenile snails obtained from a single population show markedly reduced activity and growth at increased density in the laboratory (Cameron & Carter, 1979). It seems likely that there must also be a genetic component to size variation in this species. Unfortunately several of the heritability estimates were obtained using parents from geographically widely separated populations chosen for their size differences. It is possible that these snails were from different genetic races and there is some doubt about the applicability of heritability estimates derived from such crosses to snails within local groups of populations (see Cook & Cain, 1980, for a discussion).

We have shown here that adult snails collected from less dense natural populations are more fecund by 50.8% (young/snail data) than adults of the same size range from a relatively crowded population. Further, the sparse population provided some snails which were larger than any from the dense population and these were most fecund under our experimental conditions. The dense population provided the group of smallest snails which were least fecund under the same experimental conditions.

We know (Carter, Ashdown & Morgan-Huws, in preparation) that mean shell sizes in these populations are inversely related to density and that the mean sizes have varied in a regular way in recent years. Thus, although there may be genetic differences underlying differences in shell size there is good evidence that density differences cause substantial variation in this character and in fecundity as well. The effects of density that have been observed in experiments would encourage population expansion when adult densities were low and population contraction when densities were high.

We cannot be certain which mechanism is

bringing about the density effects. However, it seems unlikely that food was limiting in either the growth or the fecundity experiments. Competition for egg laying sites may limit fecundity but interaction between snails by means of mucus which has been shown to occur in this and other pulmonate gastropods (Cook, 1977; Chase, Pryer, Baker & Madison, 1978; Cameron & Carter, 1979; Dan & Bailey, 1982) would seem to be important in limiting both growth and fecundity in *Cepaea nemoralis.*

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REFERENCES CITED

BERRIE, A. D. & VISSER, S. A., 1963, Investigations of a growth-inhibiting substance affecting a natural population of freshwater snails. *Physiological Zoology,* 36: 167–173.

BUTLER, A. J., 1976, A shortage of food for the terrestrial snail *Helicella virgata* in South Australia. *Oecologia (Berlin),* 25: 349–371.

CAIN, A. J. & CURREY, J. D., 1968, Studies on *Cepaea* III. Ecogenetics of a population of *Cepaea nemoralis* (L.) subject to strong area effects. *Philosophical Transactions of the Royal Society of London,* ser. B, 253: 447–482.

CAMERON, R. A. D. & CARTER, M. A., 1979, Intra- and inter-specific effects of population density on growth and activity of some helicid land snails (Gastropoda: Pulmonata). *Journal of Animal Ecology,* 48: 237–246.

CARTER, M. A., ASHDOWN, M. & MORGAN-HUWS, D. I., in preparation, Environmental variation, snail density and shell phenotype variation in a group of *Cepaea nemoralis* (L.) populations in southern England.

CHASE, R., PRYER, K., BAKER, R. & MADISON, D., 1978, Responses to chemical stimuli in the terrestrial snail *Achatina fulica* (Pulmonata: Sigmurethra). *Behavioural Biology,* 22: 302–315.

COOK, A., 1977, Mucus trail following by the slug *Limax grossui* Lupu. *Animal Behaviour,* 25: 774–781.

COOK, L. M. & CAIN, A.J., 1980, Population dynamics, shell size and morph frequency in experimental populations of the snail *Cepaea nemoralis* (L.) *Biological Journal of the Linnean Society,* 14: 259–292.

DAN, N. A. & BAILEY, S. E. R., 1982, Growth, mortality and feeding rates of the snail *Helix aspersa* at different population densities in the laboratory and the depression of activity of helicid snails by other individuals or their mucus. *Journal of Molluscan Studies,* 48: 257–265.

EISENBERG, R. M., 1966, The regulation of density in a natural population of the pond snail *Lymnaea elodes. Ecology,* 47: 889–906.

EISENBERG, R. M., 1970, The role of food in the regulation of the pond snail *Lymnaea elodes. Ecology,* 51: 680–684.

HERZBERG, F., 1965, Crowding as a factor in growth and reproduction of *Helix aspersa. American Zoologist,* 5: 354.

MOOIJ-VOGELAAR, J. W., JAGER, J. C. & VAN DER STEEN, W. J., 1970, The effect of density changes on the reproduction of the pond snail *Lymnaea stagnalis* (L.). *Netherlands Journal of Zoology,* 20: 279–288.

MOOIJ-VOGELAAR, J. W., JAGER, J. C. & VAN DER STEEN, W. J., 1973, Effects of density levels and changes in density levels on reproduction, feeding and growth in the pond snail *Lymnaea stagnalis* (L.). *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen,* ser. C, 76: 245–256.

MOOIJ-VOGELAAR, J. W. & VAN DER STEEN, W. J., 1973, Effects of density on feeding and growth in the pond snail *Lymnaea stagnalis* (L.). *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen,* ser. C, 76: 61–68.

MURRAY, J., 1964, Multiple mating and effective population size in *Cepaea nemoralis. Evolution,* 18: 283–291.

OOSTERHOFF, L., 1977, Variation in growth rate as an ecological factor in the landsnail *Cepaea nemoralis* (L.). *Netherlands Journal of Zoology,* 27: 1–132.

PELLUET, D. & LANE, N. J., 1961, The relation between neurosecretion and cell differentiation in the ovotestis of slugs (Gasteropoda: Pulmonata). *Canadian Journal of Zoology,* 39: 789–805.

POMEROY, D. E., 1969, Some aspects of the ecology of the land snail *Helicella virgata* in South Australia. *Australian Journal of Zoology,* 17: 495–514.

PRICE, D. J. & BANTOCK, C. R., 1975, Marginal populations of *Cepaea nemoralis* (L.) on the Brendon Hills, England. II Variation in chiasmata frequency. *Evolution,* 29: 278–286.

THOMAS, J. D. & BENJAMIN, M., 1974, The effect of population density on growth and reproduction of *Biomphalaria glabrata* (Say) (Gasteropoda: Pulmonata). *Journal of Animal Ecology,* 43: 31–50.

THOMAS, J. D., GOLDSWORTHY, G. J. & ARAM, R. H., 1975, Studies on the chemical ecology of snails: the effect of chemical conditioning by adult snails on the growth of juvenile snails. *Journal of Animal Ecology,* 44: 1–28.

THOMAS, J. D., GOLDSWORTHY, G.J. & BEN-

JAMIN, M., 1974, Chemical conditioning of the environment by the fresh water pulmonate snail *Biomphalaria glabrata* and its effect on growth and natality rates. *Journal of Zoology (London),* 172: 443–467.

VIANEY-LIAUD, M., 1979, Influence du jeûne et de la renutrition sur l'oviposition et les gamétogenèses chez le planorbe *Biomphalaria glabrata* (Gastéropode Pulmoné Basommatophore). *Malacologia,* 18: 401–406.

WILLIAMSON, P., 1979, Age determination of juvenile and adult *Cepaea. Journal of Molluscan Studies,* 45: 52–60.

WILLIAMSON, P. & CAMERON, R. A. D., 1976, Natural diet of the landsnail *Cepaea nemoralis. Oikos,* 27: 493–500.

WILLIAMSON, P., CAMERON, R. A. D. & CARTER, M. A., 1976, Population density affecting adult shell size of snail *Cepaea nemoralis* L. *Nature,* 263: 496–497.

WOLDA, H., 1963, Natural population of the polymorphic land snail *Cepaea nemoralis* (L.). *Archives Néerlandaises de Zoologie,* 15: 381–471.

WOLDA, H., 1965, The effect of drought on egg production in *Cepaea nemoralis* (L.). *Archives Néerlandaises de Zoologie,* 16: 387–399.

WOLDA, H., 1967, The effect of temperature on reproduction in some morphs of the land snail *Cepaea nemoralis. Evolution,* 21: 117–129.

WOLDA, H. & KREULEN, D., 1973, Ecology of some experimental populations of the land snail *Cepaea nemoralis* (L.). II. Production and survival of eggs and juveniles. *Netherlands Journal of Zoology,* 23: 168–188.

WRIGHT, C. A., 1960, The crowding phenomenon in laboratory colonies of fresh water snails. *Annals of Tropical Medicine and Parasitology,* 54: 224–232.

YOM-TOV, Y., 1972, Field experiments on the effect of population density and slope direction on the reproduction of the desert snail *Trochoidea (Xerocrassa) seetzeni. Journal of Animal Ecology,* 41: 17–22.

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A TRANSPLANTATION EXPERIMENT ON TWO SPECIES OF HELICID SNAILS IN NORTHERN SCOTLAND

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ABSTRACT

Helicid snails have a patchy distribution in northern Scotland being confined to dunes and grassland on shell-sand. Such habitat forms a series of isolated areas, so snail populations are confined to a set of ecological islands. Species that are present in one locality may be absent from others. The same is true of individual morphs of polymorphic species. Such a pattern of dispersion may result from the exigencies of colonization and extinction. Alternatively, it may result from the ecological differences between sites, if ecological factors determine which species and morphs survive at each site. This paper describes an experimental study that was aimed at discovering the relative importance of these two possible causes.

The species studied were *Cepaea hortensis* and *Arianta arbustorum.* Collections of adults were made at two sites. The sites differed in topography, vegetation, and population density of the snails. One site, Strathy, had both species. The *C. hortensis* comprised both banded and unbanded individuals but all were yellow in colour. The other site, Torrisdale, only had *C. hortensis.* All of them were banded, though browns were present as well as yellows. The collected snails were marked. Half were returned to their native site and half were transplanted to the other site. A year later both marked and unmarked snails were collected. From the proportions of the marked and unmarked individuals found alive and dead it was possible to draw some conclusions about the relative survival rates of different morphs and of the two species. There were difficulties in distinguishing differences in survival from differences in behaviour, which we discuss. From the proportion of marked snails found outside the original study plots, we could draw some conclusions about emigration rates.

In *C. hortensis,* snails from Torrisdale emigrated from both study plots more than did snails from Strathy. *C. hortensis* probably survived better at Strathy than at Torrisdale and also probably survived better if they originated from Strathy rather than from Torrisdale, these two effects being independent. Unbanded snails survived better relative to banded at Torrisdale than at Strathy. However, there was evidence of between-year differences in the relative survival rates of the two morphs.

The results gave clear evidence of difference in activity patterns beween the species. There may have been differences in survival rate but these are confounded with probable differences in activity patterns.

Predation by birds varied in intensity from year to year and from place to place. The relative rate of predation on the two species also differed.

The experiment failed to provide a clear answer to our original question. Such experiments are probably only worth carrying out if they involve many pairs of sites that are observed intensively for periods greater than one year. There may be better ways of discovering the relative importance of chance and of systematic ecological features in determining the composition of snail faunas.

Key words: *Cepaea; Arianta;* polymorphism; selection; predation; biogeography; isolation.

INTRODUCTION

In northwest Scotland helicid snails are almost entirely restricted to isolated areas of shell-sand close to the sea. This paper records an attempt to determine whether the isolation of populations is important in determining their specific and genetic compositions. Snails were collected from two plots, one on each of two isolated dune systems some 15 km apart. After marking, half were transferred to the other plot and half were replaced on their native ground. Repeat collections were made a year later to compare the fates of transferred and native snails.

Cain, Cameron & Parkin (1969) recorded the variation of *Cepaea hortensis, Arianta arbustorum* and *Helicella itala* in the same

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region. We used their work to plan our experiments but confined our attention to the first two species.

METHODS AND MATERIALS

General

Snails were collected from two plots on two successive days in 1969. The individuals of each morph that were collected on each day at each site were randomly divided into two groups of equal number. After the second day's collecting one of these was released in its native plot; the other was transferred to the other plot. All snails were marked according to their origin. One year later both marked and unmarked snails were collected from the plots and from the immediately surrounding areas. The information so obtained allowed us to study differences in rates of survival and dispersal between snails of different species, between snails of different morphs, between snails from different places, and between snails at different places. The native populations in the two plots differed in both specific and genetic composition and we anticipated that we might observe selection against the species or morphs absent from each site.

Collecting, marking and releasing

In August 1969, study plots were marked by driving stakes into the ground. From each plot snails were collected on two successive days by one of us searching in a series of parallel traverses, with the other searching traverses at right angles. A similar method was employed in September 1970, but we worked for three days and were assisted by Mr. R. M. Blindell, who searched in parallel to one of us but independently. On the second day in 1970 we also searched a strip ten metres wide around the edge of each plot by walking side-by-side around the periphery in an increasing spiral. Most collecting was done between 05.00 and 09.00 G.M.T., a time at which the snails were active. Table 1 summarizes the snails found.

When collecting in 1969, we removed all adult snails and dead juveniles. The dead shells were retained; the live adults were marked and either replaced or transferred. Marking was by drilling a hole behind the lip of the shell: we have no reason to suppose that this affected the viability of the snail. All the snails recaptured alive in 1970 and 87% of the dead ones had sealed the hole with calcareous unpigmented material, the outer surface of which was slightly below the level of the shell. Such marks will persist as long as the shell and there is no difficulty in detecting them. Since the mark is close to the lip it is rarely destroyed when the shell is smashed by a predator.

The snails were collected on 3 and 4 August at Strathy and 4 and 5 August at Torrisdale. They were kept in cardboard boxes until being released on 5 August between 06.00 and 07.30 G.M.T., in humid conditions when local snails were active. We released the marked animals by walking over the plots in a grid pattern and dropping them at regular intervals.

The plots and their snails

Strathy (National Grid Reference NC840659)

The plot was 20 metres square. It comprised stabilised sand dunes, slightly undulating, and with one deep pit. About half the vegetation was marram (*Ammophila arenaria*). The ground was largely covered with bryophytes.

Both *C. hortensis* and *A. arbustorum* were found here. In 1969, live adults of the two species had population densities of $2.3/m^2$ and $0.35/m^2$ respectively.

Torrisdale (NC687616)

This plot was 40 metres square. It comprised stabilised sand dunes in the form of steep hummocks separated by pits and channels. The tops of the hummocks were 3 to 7 metres above the level of the pits. The sand was exposed over much of the area. Marram and other herbs were sparse, most of the vegetation being bryophyte heath.

A. arbustorum was not found here. Live adult *C. hortensis* had a population density of $0.04/m^2$ in 1969.

Genetic variants studied

In *C. hortensis* one locus governs shell color, with complete dominance of brown over pink and of pink over yellow. Closely linked to this, a second locus controls banding, absence being dominant to presence (Cook & Murray, 1966). Pinks were absent from the populations that we studied. The brown shells were very pale, so that some of the shells

TABLE 1. The numbers of various morphs of *Cepaea hortensis* and *Arianta arbustorum* collected, transplanted, and re-collected at Strathy and Torrisdale in 1969 and 1970. Only live adults were marked. ? Indicates color unscorable.

			Collected 1969			Marked 1969		Collected 1970															
								Adults inside plots						Adults around plots						Juveniles			
			Adults					Marked natives		Marked introduced		Unmarked		Marked natives		Marked introduced		Unmarked		Inside		Around	
		Species and morph	live	dead	juvenile (dead)	replaced	transferred	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
STRATHY	*C. hortensis*	Yellow unbanded	403	120	15	202	201	73	19	—	—	488	119	14	1	—	—	386	99	316	42	118	14
		Yellow banded	547	240	25	273	274	102	14	2	2	785	168	23	3	2	1	565	187	387	48	150	31
		Brown banded	0	0	0	—	—	—	—	3	1	0	0	—	—	1	1	0	0	0	0	0	0
		? banded	0	0	0	—	—	—	—	1	0	0	0	—	—	1	0	0	0	0	0	0	0
	A. arbustorum	Yellow unbanded	6	3	1	3	3	1	0	—	—	1	0	0	0	—	—	0	0	0	0	0	0
		Brown banded	133	99	30	66	67	6	6	—	—	124	54	1	0	—	—	30	64	11	33	3	25
		Brown unbanded	3	0	0	2	1	0	0	—	—	0	0	0	0	—	—	0	0	0	0	0	0
TORRISDALE	*C. hortensis*	Yellow unbanded	0	0	0	—	—	—	—	44	37	0	0	—	—	15	2	0	0	0	0	0	0
		Yellow banded	29	8	0	14	15	2	4	45	45	19	13	2	2	4	5	13	2	10	3	4	1
		Brown banded	22	6	0	10	11	5	0	—	—	18	10	1	0	—	—	11	2	2	1	1	0
		? banded	9	13	1	5	4	0	4	—	—	5	19	0	0	—	—	0	9	0	0	0	0
	A. arbustorum	Yellow banded	0	0	0	—	—	—	—	0	0	0	0	—	—	0	0	0	0	0	0	0	0
		Brown banded	0	0	0	—	—	—	—	3	12	0	0	—	—	1	1	0	0	0	0	0	0
		Brown unbanded	0	0	0	—	—	—	—	0	0	0	0	—	—	0	0	0	0	0	0	0	0

could not be scored with certainty as either brown or yellow. The proportion of browns in the scorable shells was zero at Strathy and 45% at Torrisdale. The banded shells mostly had five unfused bands. The proportion of banded was 60% at Strathy and 100% at Torrisdale.

In *A. arbustorum* there is also a pair of linked loci governing color and banding. Brown is dominant to yellow but the absence of bands is recessive to their presence (Cook & King, 1966). There is usually only one band in this species. At Strathy, 4% of the snails were yellow unbanded, 15% brown unbanded, and 81% brown banded.

Statistical methods

Most of our statistical analyses involved comparing proportions. We have used the usual methods for contingency tables, applying the general linear model to detect higher-order interactions when appropriate.

POTENTIAL PROBLEMS OF INTERPRETATION

Collecting efficiency

The number of shells collected declined on successive days, as expected. For dead shells the data were consistent with the assumptions that all shells available for collection on the first day remained available on subsequent days (unless already captured), that no shells became available on subsequent days that had not been available on the first day, and that the same proportion of shells available on that day was collected on each day. From the negative exponential decline in numbers caught we conclude that we found over 85% (1969) and 98% (1970) of dead adult shells with little variation between sites, days, or species. We probably found over 98% (1969) and over 80% (1970) of those live adults that were active during the sampling periods, though the figures were more heterogeneous than those for dead shells. Because these estimates are based on only two or three successive collections, they are not highly reliable. Nonetheless, they suggest that we found most of the available adult shells. We were much less efficient at finding juveniles.

Selection during sampling

Since we removed a large proportion of the available snails each day, any unconscious selection by us should appear as changes in relative frequencies in successive samples. On this basis we seem not to have selected one species preferentially. However, at Strathy we did tend to select unbanded *C. hortensis* adults more than banded (Table 2). As our overall efficiency was so high it is improbable that our final totals of live or dead snails were greatly biased by the selection that we imposed.

Mortality and dispersal

We have nowhere combined data from inside and outside the plots without first checking for heterogeneity, since two forms of bias might otherwise occur. The first is that marked snails may have spent comparatively longer within the plots where they were deposited than around the periphery, so there might be a higher proportion of dead marked shells inside the plots. Secondly, since we collected around the plots only once but three times inside them and since the efficiency of sampling was perhaps higher for dead shells there might tend to be a higher proportion of dead shells in our samples from outside the plots.

TABLE 2. Changes in the proportion of unbanded *C. hortensis* between the first and subsequent days of collection. Sample sizes are given in brackets. Results for live and dead shells and for the two years have been combined, since there were no detectable heterogeneities. There is significant heterogeneity between the three groups shown in the table ($\chi^2 = 9.8$, 2 d.f., $p < 0.05$). There was no difference between days either at Torrisdale or among juveniles at Strathy ($\chi^2 = 1.0$, 1 d.f., $p = 0.5$ in each case) but there were more unbandeds among Strathy adults on the first day ($\chi^2 = 15$, 1 d.f., $p < 0.001$).

Site	Age	First day	Subsequently
Strathy	Adults	0.42 (2207)	0.34 (870)
Torrisdale	Adults	0.36 (122)	0.43 (86)
Strathy	Juveniles	0.44 (422)	0.46 (411)

Survival and availability

This makes it difficult to estimate survival rates. A simple model illustrates the problem. Let the proportion of marked snails that survive be P_s and the proportion of marked snails that are subsequently found be P_A. Let the proportions of marked snails that survive and are found be P_{LF}, that survive and are not found be P_{LN}, that die and are found be P_{DF}, and that die and are not found be P_{DN}. Our expectation is then:

$$P_{DN} = (1 - P_s)(1 - P_A) - x$$
$$P_{DF} = (1 - P_s) P_A + x$$
$$P_{LN} = P_s (1 - P_A) + x$$
$$P_{LF} = P_s P_A + x$$

where x, which may be positive or negative, is the tendency for dead shells to be found more or less readily than live ones. If live and dead are found equally readily, x will be zero.

Our observations provide direct estimates of P_{DF} and P_{LF} (and thus of P_A) but only of the sum of P_{DN} and P_{LN}, so that x and P_s cannot be estimated. The best we can do is to calculate a 'live index':

$$P_{LF}/(P_{LF} + P_{DF}) = P_s - x/P_A \qquad (1)$$

However, our purpose in this paper is not to make absolute estimates of survival rate but to compare survival of different groups of snails. This is slightly easier. Suppose that two groups have the same value of P_A, which we shall call the 'availability index.' Suppose also that they have the same value of live index. From equation (1), we see that it is possible that they differ in P_s values (survival rates) but that the difference is exactly compensated by a difference in x values. However, this seems less likely than the simpler explanation that they have the same values of P_s and of x; i.e., that they have the same survival rate as well as the same pattern of availability.

Suppose that two groups have similar availability indices but dissimilar live indices. The simplest interpretation is that they differ in survival rate in the same direction as the difference in live index. It is conceivable that they have the same survival rate but differ in x value but this seems unlikely: it implies that they differ in the tendency for dead snails to be more or less available than live ones even though they do not differ in overall availability. (It should be noted that since the sample sizes for availability indices are larger than those for the corresponding live indices a non-significant difference in availability index coupled with a significant difference in live index means that their live indices really are more dissimilar than their availability indices.)

Should the two groups differ in availability index as well as in live index, a variety of interpretations is possible. In general one can only conclude that they differ in availability and may or may not differ in survival rate. However, if the difference in live index runs counter to that in availability index, the most likely explanation is that the two groups differ in survival rate as well as in availability, the differences being in opposite directions.

Reproduction of transplanted snails

Transplanted snails appear not to have contributed to the juveniles collected in 1970, for we found no juveniles of non-native forms (i.e. *A. arbustorum* and brown *C. hortensis* at Torrisdale, unbanded *C.hortensis* at Strathy). We did not expect them to do so: they are unlikely to have laid before spring 1970; juveniles of these species grow slowly; and we collected few small juveniles.

SITE-RELATED DIFFERENCES IN *C. HORTENSIS*

Dispersal

A crude measure of dispersal is the proportion of marked snails found in 1970 that was in the 10 m strip around each study plot rather than within it. The appropriate proportions for *C. hortensis* in the two populations are shown in Table 3a. The greater rate of dispersal at both places for snails from Torrisdale is significant ($\chi^2 = 6.7$, 1 d.f., $p < 0.01$). The apparently greater rate for snails at Strathy is not significant and in any case is explicable simply by the smaller size of the Strathy plot. Note that there is no second-order interaction, which would imply an effect of displacement: the difference in rate of dispersal between snails from Strathy and those from Torrisdale is the same at both places.

Survival and availability

There is a significant second-order interaction in the availability indices (Table 3b: $\chi^2 = 4.9$, 1 d.f., $p < 0.05$): snails from Strathy are

TABLE 3. Dispersal, availability, and live indices for *C. hortensis* originating from and placed at Strathy and Torrisdale. See text for explanation of indices. Sample sizes are given in brackets.

	Origin of snails	
	Strathy	Torrisdale
a) Dispersal		
At Strathy	0.16 (249)	0.40 (15)
At Torrisdale	0.13 (197)	0.25 (20)
b) Availability		
At Strathy	0.52 (475)	0.50 (30)
At Torrisdale	0.41 (475)	0.69 (29)
c) Live		
At Strathy	0.85 (249)	0.67 (15)
At Torrisdale	0.55 (197)	0.50 (20)

more available at Strathy than at Torrisdale ($\chi^2 = 23$, 1 d.f., $P < 0.001$) but this is not true for snails from Torrisdale ($\chi^2 = 0.0$, 1 d.f., $P > 0.9$) or, to put it another way, at Torrisdale snails from Torrisdale are more available than snails from Strathy ($\chi^2 = 10.2$, 1 d.f., $P < 0.01$) but this is not true at Strathy ($\chi^2 = 1.5$, 1 d.f., $P > 0.1$). The live indices show no such interaction. Instead, the indices are uniformly greater for snails at Strathy than at Torrisdale ($\chi^2 = 50$, 1 d.f., $P < 0.001$). They are also greater for snails from Strathy than from Torrisdale but the difference is not significant. The lack of difference is probably just a reflection of the small numbers from Torrisdale: if survival is measured as the proportion of all snails marked that were recovered alive (P_{LF} in terms of the formal model), the greater survival of snails from Strathy is clearly significant ($\chi^2 = 7.0$, 1 d.f., $p < 0.01$), as is that of snails from Strathy ($\chi^2 = 27$, 1 d.f. $p < 0.001$). Again, there is no second-order interaction ($\chi^2 = 0.02$, 1 d.f., $p > 0.5$).

Because there is a second-order interaction in the availability indices but not in the live indices it is unlikely that the four groups had the same survival rate. Pairwise comparisons allow more detailed conclusions as follows. At Strathy, snails from Strathy and from Torrisdale have similar availability indices but those from Strathy have the higher live index: thus snails from Strathy probably survived better. At Torrisdale, no conclusion about relative survival rates is possible because the availability index is clearly higher for snails from Torrisdale than for those from Strathy but their live index is only a little, if any, higher. Snails from Torrisdale had a higher live index at Strathy than at Torrisdale but not a higher availability index: they probably survived better at Strathy. Snails from Strathy had a somewhat higher availability index at Strathy than at Torrisdale but a much higher live index, suggesting that they probably survived better. Overall, it seems probable that *C. hortensis* probably survived better at Strathy than at Torrisdale and if they came from Strathy than if they came from Torrisdale.

The only morph that was reciprocally transplanted was yellow banded. Results for this morph alone are similar to those for all morphs combined, though statistically less significant. Yellow unbandeds, all from Strathy, survived better there than at Torrisdale ($p < 0.01$). Browns, all from Torrisdale, may have survived better there, but the difference is not significant.

DIFFERENCES BETWEEN MORPHS OF *C. HORTENSIS*

Dispersal

Our results provide no evidence of differences between morphs in dispersal rate.

Survival and availability

The small number of snails from Torrisdale and the difficulty of distinguishing yellows from browns mean that morph-related differences in survival could only be detectable if very large. They were not.

The *C. hortensis* population from Strathy comprised both banded and unbanded snails. As shown in Table 4a, the latter had a higher availability index than the former ($\chi^2 = 4.3$, 1 d.f., $p < 0.05$), there being no heterogeneity between the two plots in this effect ($\chi^2 = 3.3$, 1 d.f., $p > 0.05$). This is consistent with our tendency to select unbandeds at Strathy but we had no such tendency at Torrisdale (Table 2). Furthermore, as we have argued above, we collected such a high proportion of the available snails that our overall totals are unlikely to have been biased by this selection. This is confirmed by the proportion of marked snails among the natives collected at Strathy in 1970 being almost the same among banded and unbanded (0.082 and 0.083): if our selection biased the overall totals this proportion should have been higher among

TABLE 4. Availability and live indices for *C. hortensis* from Strathy. Sample sizes are given in brackets.

	Location	
	Strathy	Torrisdale
a) Availability		
Banded	0.52 (273)	0.36 (274)
Unbanded	0.53 (202)	0.49 (201)
b) Live		
Banded	0.88 (142)	0.49 (99)
Unbanded	0.81 (107)	0.60 (98)

TABLE 5. Proportion of live snails among adult native *C. hortensis* at Strathy. Sample sizes are given in brackets.

	Banded	Unbanded
1969	0.70 (787)	0.77 (523)
1970 (Unmarked)	0.79 (1705)	0.80 (1092)
1970 (Marked)	0.88 (142)	0.81 (107)
1970 (All)	0.80 (1847)	0.80 (1199)

unbanded. It is probable, therefore, that unbanded shells are genuinely more available than bandeds in 1970.

If we turn to live indices (Table 4b) we find a different picture, with significant heterogeneity between plots ($\chi^2 = 4.4$, 1 d.f., $p < 0.05$). Thus, even allowing for differences in availability there must be differences in survival rate: unbandeds survived bettter relative to banded at Torrisdale than at Strathy.

Unmarked snails provide further evidence of differences in survival at Strathy. Considering only natives, the proportion of live individuals was almost identical among unbandeds and bandeds in 1970 but was lower among bandeds in 1969 (Table 5). Statistical analysis is complicated by a small but significant difference between unmarked and marked snails in 1970 (Table 5: $\chi^2 = 4.4$, 1 d.f., $p < 0.05$). However, the heterogeneity between years in the morph-related difference is significant whether one compares 1969 with unmarked 1970 snails or with marked ones ($\chi^2 = 4.3$ and 5.8 respectively; 1 d.f.; $p < 0.05$ in each case). This suggests that morph-related differences in survival during 1969/1970 were different from what they had been, on average, for some years prior to 1969, or that morph-frequencies were changing during those years, or both.

There is no evidence that the morph-related differences in survival at Strathy varied according to the age of the snails. For example, the proportion of live individuals, though higher in juveniles than adults, was no higher in banded juveniles than in unbandeds. (89% of 490 and 87% of 616 respectively.)

The incidence of predation may be studied from the shell fragments left by predators (see below). There is no evidence that predation rates in *C. hortensis* were morph-related.

Reproduction

The proportion of unbanded shells among juveniles at Strathy in 1970 was almost identical to that among live adults in 1969, suggesting that any changes in morph-frequency between generations and any morph-related differences in reproductive rate are small.

DIFFERENCES BETWEEN SPECIES

Dispersal

Our results provide no evidence of differences between species in dispersal rate.

Differences in capture rate according to time of day

Of all the samples we made, seven had an excess of *A. arbustorum* compared with the total of all the samples from the site in question, while eight had an excess of *C. hortensis.* Those with an excess of *A. arbustorum* were taken earlier in the day, on average, than those with an excess of *C. hortensis* ($p < 0.01$, Wilcoxon-Mann-Whitney U-test). Since both species became less active as the morning wore on, this difference may have been as a result of *A. arbustorum* retiring earlier or of it being relatively less conspicuous when resting.

Survival and availability

The availability indices for snails originating from Strathy (Table 6a), while indicating that *C. hortensis* was more available than *A. arbustorum* at both sites (Strathy, $\chi^2 = 50$, 1 d.f., $p < 0.001$; Torrisdale, $\chi^2 = 7.2$, 1 d.f., $p < 0.01$), show that the difference between the species was greater at Strathy ($\chi^2 = 8.8$,

TABLE 6. Availability and live indices for snails from Strathy. Sample sizes are given in brackets.

	Location	
	Strathy	Torrisdale
a) Availability		
C. hortensis	0.52 (475)	0.41 (475)
A. arbustorum	0.20 (71)	0.24 (71)
b) Live		
C. hortensis	0.85 (249)	0.55 (197)
A. arbustorum	0.57 (14)	0.24 (17)

TABLE 7. Proportion of live snails among adult natives at Strathy. Sample sizes are given in brackets.

	C. hortensis	*A. arbustorum*
1969	0.73 (1310)	0.58 (246)
1970 (Unmarked)	0.80 (2797)	0.57 (272)
1970 (Marked)	0.85 (249)	0.58 (14)

TABLE 8. Proportion of live snails among adult and juvenile native *C. hortensis* at Strathy in 1970. Sample sizes are given in brackets.

	Adults	Juveniles
C. hortensis	0.80 (3046)	0.88 (1106)
A. arbustorum	0.57 (286)	0.19 (72)

1 d.f., $p < 0.01$). The live indices (Table 6b) show no such second order interaction but greater values overall for *C. hortensis* ($\chi^2 = 12.4$, 1 d.f., $p < 0.001$) and for both species at Strathy ($\chi^2 = 54$, 1 d.f., $p < 0.001$).

Despite the rather different patterns displayed by the availability and live indices, the magnitude of the differences in availability is such that the live indices are probably poor measures of survival and may merely reflect differences in availability. It would therefore be unwise to conclude that rates of survival were different in the two species. (Note that the apparently better survival of *A. arbustorum* at Strathy than at Torrisdale, with a large difference in live index contrasting with almost none in availability index, is not significant, sample sizes being very small.)

Differences between the species in either activity rates or survival rates are confirmed by considering the proportion of live snails among unmarked shells at Strathy, which are similar to those among marked shells (Table 7). The excess of live shells in *C. hortensis* is significant for each of the three rows in the table ($p < 0.05$ in each case). That the species differences are age-related is indicated by the different pattern shown by the proportion of live shells in adults and juveniles (Table 8: second-order interaction: $\chi^2 = 56$, 1 d.f., $p < 0.0001$).

PREDATION

Some invertebrate predators eat snails without damaging the shells and some vertebrates swallow them whole or smash the shells to small fragments. But others break the shells in such a way that the cause of death is obvious from inspecting the shell alone, especially in the case of the song thrush *Turdus ericetorum,* which has a special method of breaking the shells (Cain & Sheppard, 1954; Morris, 1954). Most of the predator-smashed shells found in our plots had been broken by thrushes. They amounted to 0–21% of the dead shells, with considerable variation between sites and species (Table 9).

The data for *C. hortensis* found inside each plot show that the predation rate was significantly higher at Strathy than at Torrisdale ($\chi^2 = 5.3$, 1 d.f., $p < 0.05$) and higher in 1969 than in 1970 ($\chi^2 = 10.3$, 1 d.f., $p < 0.01$). There was no interaction between sites and years, but this is scarcely to be expected, given the low level of predation overall at Torrisdale. The data for 1970 at Strathy show no significant difference betwewen the species ($\chi^2 = 0.02$, 1 d.f., $p > 0.5$) but a significant difference between the plot and its immediate surroundings ($\chi^2 = 33$, 1 d.f., $p < 0.001$), with no interaction between species and location. The comparison is somewhat confused because the strip around the plot was not collected in 1969, so the 1970 sample may contain snails from that year. However, the proportion of depredated shells in the sample from around the plot was not only higher than that of the within-plot 1970 sample but also than that of the within-plot 1969 sample, though not significantly ($\chi^2 = 2.5$, 1 d.f., $p > 0.1$), suggesting that predation really was more intense around the plot. Finally, the data for the two years within the

TABLE 9. Proportion of dead shells that had been broken by predators. 'Inside' refers to shells collected within the plots, 'around' to those collected within 10 m of the plots. Sample sizes are given in brackets.

	1969 inside	1970 inside	1970 around
Strathy			
C. hortensis	0.17 (360)	0.06 (385)	0.21 (287)
A. arbustorum	0.08 (102)	0.10 (60)	0.14 (64)
Torrisdale			
C. hortensis	0.04 (27)	0.0 (132)	0.0 (22)
A. arbustorum	— (0)	0.0 (12)	0.0 (1)

Strathy plot show a significant heterogeneity between years in the relative rates of predation on the two species ($\chi^2 = 5.9$, 1 d.f., $p < 0.05$).

DISCUSSION

Negative results

Survival, measured in terms of live index, was not *significantly* higher in *A. arbustorum* at Strathy than at Torrisdale, the site from which the species was naturally absent. But there was a fairly large *apparent* difference: the lack of significance is probably due merely to small numbers. This illustrates an important problem in this study; numbers were so small that only large differences between groups of snails would be significant. Thus failure to demonstrate a difference merely means that any such difference was not very large.

Differences between *C. hortensis* populations

C. hortensis survived better at Strathy than at Torrisdale and snails from Strathy survived better at both sites than those from Torrisdale. The better survival at Strathy is not surprising: our transfers reduced the frequency of adults there to about 70% of its natural level, whereas they increased the frequency at Torrisdale about seven-fold. Our data do not allow us to reach any conclusions about relative survival rates at natural densities. Since the inferiority of snails from Torrisdale was manifest even at Torrisdale, it seems unlikely that it was genetic in origin, though this is not impossible.

One might have expected some tendency for snails to survive bettter where native than where transplanted. This would have been manifest in a heterogeneity between plots in the relative survival of snails from the two plots. Since there was no detectable heterogeneity, any effect of being on home ground must have weaker than the major effects of being at and coming from Strathy.

The fact the *C. hortensis* from Torrisdale migrate more than those from Strathy agrees with other studies in *Cepaea* which indicate that snails from different populations may move to different extents (Wolda, 1963), and that, in particular, *Cepaea* is more mobile when the density is low (Greenwood, 1975; Oosterhoff, 1977; Cameron & Carter, 1979). This may be an adaptation to a sparse food supply or to finding potential mates sufficiently often. Since movement of snails from the same origin was not obviously different at the two sites the higher mobility of the Torrisdale snails might be a genetic or developmental effect which is not readily susceptible to short-term modification.

This study is the first in which differences in survival and dispersal have been demonstrated between *C. hortensis* from different places although such differences are known in *C. nemoralis* (Wolda, 1963; Cook & Cain, 1980).

Differences between morphs of *C. hortensis*

There are various known selective differences between the morphs of *Cepaea* (see reviews by Cain, 1977; Jones, Leith & Rawlings, 1977; Clarke *et al.*, 1978). In our experiments differences in survival rate were somewhat obscured by differences in availability but the survival of unbandeds relative to that of bandeds appears to have been better at Torrisdale than at Strathy during 1969/70. This suggests that the absence of

unbandeds from Torrisdale is a mere historical accident. However, one year's results are scarcely enough: our own data suggest that selection at Strathy prior to 1969 was different from that during 1969/70 and we would be surprised if any selective values remained constant from year to year. Thus our experiment does not allow us to favour either selection or historical accident as the cause of the absence of particular morphs from sites.

We found unbanded adults more readily than bandeds at Strathy. The latter may have been better camouflaged or they may have been less active at the times we were searching. We have argued that we collected such a high proportion of the available snails that such selection cannot alone have been responsible for the greater 'availability indices' of unbandeds, but that the unbandeds were genuinely more available at the season in which we collected. Since the difference involved dead shells as well as live snails, it seems probable that unbandeds had a general tendency to spend less of their life hidden in litter or buried in sand than bandeds.

Differences between species

C. hortensis was more available than *A. arbustorum,* especially later in the mornings. This suggests that it was more diurnal since both species were harder to find when inactive, when they nestled into the vegetation and litter. Sand dunes, though they are often humid by night, tend to be dry by day, so the more retiring habit of *A. arbustorum* is not surprising, for the species tends to be found in damper sites than the *Cepaea* spp. (Cain, Cameron & Parkin, 1969; Cameron, 1969b, 1970a; Cameron & Palles-Clark, 1971). Indeed, experiments show that it loses more water when active than do the *Cepaea* spp. (Cameron, 1970b). However, the same experiments showed that *A. arbustorum* was more diurnal than *Cepaea* in conditions of constant, high humidity and that it became inactive less readily under low humidity, which does not fit in with our results. The discrepancy may indicate that our snails were reacting to variables such as temperature that were constant in the laboratory experiments. Alternatively, these behavioral traits may be population-specific, rather than species-specific: the experimental snails came from tall vegetation in a damp, sheltered site, where humidity probably varies less rapidly than on the sand dunes.

Unfortunately, the differences in availability completely obscured any differences that there may have been in survival rate. The age-related differences demonstrable between the species could have resulted from differences in behavior, survival, or both. Thus our experiment does not allow us to favor either present ecology or historical accident as the cause of the absence of *A. arbustorum* from Torrisdale.

Predation

The rate of predation differed between sites and between years. Such differences are well-known (Goodhart, 1958; Davies & Snow, 1965; Cameron, 1969a). In addition, there may have been a difference in rate between the Strathy plot and the strip 10 m around it, presumably reflecting the habits of one or a few thrushes: one of the commonest findings in studies of vertebrate predators is that there is a great variation in behavior between individuals. Thus, to establish general conclusions one requires more extensive data than we obtained. It is interesting to note, however, the apparent absence of between-morph or between-species selection by the thrushes in this study. After all, we selected unbanded adults at Strathy and others have shown that thrushes prey selectively on the morphs of *C. nemoralis* (Sheppard, 1951; Goodhart, 1968; Wolda, 1963; Carter, 1968). Furthermore *C. hortensis* was more available to us than was *A. arbustorum* and the relative rates of predation on the two species varied seasonally at a site in England (Cameron, 1969a).

Lessons concerning technique

Jones & Parkin (1977) used the transplant technique in studies of *C. vindobonensis.* They used more plots but fewer snails per plot. They also failed to obtain conclusive results. From their experience and ours, it seems reasonable to conclude that such experiments are only worth carrying out if many pairs of sites are used, with large numbers of snails at each, and if the populations are observed intensively and for longer than one year. It may be more efficient to attempt to identify the potentially important selective factors acting on the populations and to test the significance of these in carefully controlled conditions.

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LITERATURE CITED

CAIN, A. J., 1977, The efficacy of natural selection in wild populations. In GOULDEN, C. E., ed., *The Changing Scenes in Natural Sciences 1776–1976. Academy of Natural Sciences of Philadelphia Special Publication* 12: 111–113.

CAIN, A. J., CAMERON, R. A. D. & PARKIN, D. T., 1969, Ecology and variation of some helicid snails in northern Scotland. *Proceedings of the Malacological Society of London,* 38: 269–299.

CAIN, A. J. & SHEPPARD, P. M., 1954, Natural selection in *Cepaea. Genetics,* 39: 89–116.

CAMERON, R. A. D., 1969a, The distribution and variation of three species of land snail near Rickmansworth, Hertfordshire. *Zoological Journal of the Linnaean Society of London,* 48: 83–111.

CAMERON, R. A. D., 1969b, Predation by song thrushes *Turdus ericetorum* (Turton) on the snails *Cepaea hortensis* (Mull.) and *Arianta arbustorum* (L.). near Rickmansworth. *Journal of Animal Ecology,* 38: 547–553.

CAMERON, R. A. D., 1970a, Differences in the distributions of three species of helicid snail in the limestone district of Derbyshire. *Proceedings of the Royal Society of London,* ser. B, 176: 131–159.

CAMERON, R. A. D., 1970b, The survival, weight-loss and behaviour of three species of land snail in conditions of low humidity. *Journal of Zoology, London,* 160: 143–157.

CAMERON, R. A. D. & CARTER, M. A., 1979, Intra- and interspecific effects of population density on growth and activity in some helicid land snails (Gastropoda: Pulmonata). *Journal of Animal Ecology,* 48: 237–246.

CAMERON, R. A. D. & PALLES-CLARK, M. A., 1971, *Arianta arbustorum* on chalk downs in southern England. *Proceedings of the Malacological Society of London,* 39: 311–318.

CARTER, M. A., 1968, Thrush predation of an experimental population of the snail *Cepaea nemoralis* (L.). *Proceedings of the Linnaean Society of London,* 179: 241–249.

CLARKE, B., ARTHUR, W., HORSLEY, D. T. & PARKIN, D. T., 1978, Genetic variation and natural selection in pulmonate molluscs. In *Pulmonates,* Vol. 2A, FRETTER, V. & PEAKE, J., ed., p. 219–270. Academic Press, London and New York.

COOK, L. M. & CAIN, A. J., 1980, Population dynamics, shell size and morph frequency in experimental populations of the snail *Cepaea nemoralis* (L.). *Biological Journal of the Linnaean Society of London,* 14: 259–292.

COOK, L. M. & KING, J. M. B., 1966, Some data on the genetics of the shell-character polymorphism in the snail *Arianta arbustorum. Genetics,* 53: 415–425.

COOK, L. M. & MURRAY, J., 1966, New information on the inheritance of polymorphic characters in *Cepaea hortensis. Journal of Heredity,* 57: 245–247.

DAVIES, P. W. & SNOW, D. W., 1965, Territory and food of the song thrush. *British Birds,* 58: 161–175.

GOODHART, C. B., 1958, Thrush predation on the snail *Cepaea hortensis. Journal of Animal Ecology,* 27: 47–57.

GREENWOOD, J. J. D., 1975, Effective population numbers in the snail *Cepaea nemoralis. Evolution,* 28: 513–526.

JONES, J. S., LEITH, B. H. & RAWLINGS, P., 1977, Polymorphism in *Cepaea*—a problem with too many solutions? *Annual Review of Ecology and Systematics,* 8: 109–143.

JONES, S. J. & PARKIN, D. T., 1977, Experimental manipulation of some snail populations subject to climatic selection. *American Naturalist,* 111: 1014–1017.

MORRIS, D., 1954, The snail-eating behaviour of thrushes and blackbirds. *British Birds,* 47: 33–49.

OOSTERHOFF, L., 1977, Variation in growth rate as an ecological factor in the landsnail *Cepaea nemoralis* (L.). *Netherlands Journal of Zoology,* 27: 1–132.

SHEPPARD, P. M., 1951, Fluctuations in the selective value of certain phenotypes in the polymorphic land snail *Cepaea nemoralis* (L.). *Heredity,* 5: 125–134.

WOLDA, H., 1963, Natural populations of the polymorphic land snail *Cepaea nemoralis* (L.). *Archives Néerlandaises de Zoologie,* 15: 381–471.

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POPULATION ECOLOGY OF *CEPAEA NEMORALIS* AND *C. VINDOBONENSIS* ALONG THE NORTH ADRIATIC COASTS OF ITALY

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ABSTRACT

There are sparse and discontinuous populations of *C. nemoralis nemoralis* along the north Adriatic coasts of Italy between Ravenna and Monfalcone, and of *C. vindobonensis* from the mouth of the river Tagliamento to Monfalcone.

Typically, both species live on the sand dunes that lie between the sea and the lagoons or recently drained marshes. These dunes form a series of isolated thermophilous biotopes, and were originally consolidated by *Quercus ilex*, which is now often replaced by pinewood plantations, mainly of *Pinus pinea*.

In this woodland habitat *C. nemoralis* is mainly represented by the 12345 morph, with the bands seldom fused. Pink shells are more frequent towards the north, along the Friuli coast, where there is also a richer shell-banding polymorphism. Albinos are rare, and both interrupted ("dotted") and pale ("smudged") bands are uncommon, as compared with sharply defined and continuous bands.

Average shell size increases slightly towards the south, where the climate is drier and less oceanic, with more variation between summer and winter, and where the lime content of the soil is reduced.

In *C. vindobonensis*, pale-banded shells, and shells with pale peristome lips, are rare, but they seem to occur in particularly dry habitats.

A few cases of visual predation are reported.

Key words: *Cepaea*; polymorphism; population ecology; Adriatic coasts.

INTRODUCTION

Cepaea nemoralis (L.) is not a common species along the Italian coasts of the Adriatic Sea. South of the great pinewoods of Ravenna it is no longer present, while its distribution reaches a far more southern latitude along the tyrrhenian coasts, which are moister and relatively less disturbed by human settlements (Sacchi, 1980).

While in the hinterland of the Venetian and Aemilian provinces *C. nemoralis* often forms large populations (Cesari & Orlandini, 1982) the maritime ones are usually small and discontinuous. Comparison of Figs. 1 and 2, the former showing all of the stations investigated during ten years research on land-snail ecology, the latter the reduced number of sites where *C. nemoralis* (in Friuli, also *C. vindobonensis* (Fér.)) was found, clearly confirms this statement. The letter "M" near Monfalcone (Fig. 2) marks the area where variations of *C. nemoralis* were previously studied by Piersanti (1926). Unfortunately, Piersanti's system of band classification was very peculiar and it does not enable us to use his data. The letter "R" south of Ravenna marks the district where Cesari & Orlandini (1982) are now studying, by modern methods, the polymorphism of *C. nemoralis*, largely through collections of shells killed by pinewood fires.

THE ENVIRONMENT

C. nemoralis is typically found on littoral dunes which, to a remarkable extent, due to successive shiftings of the shore lines towards the sea, border the Po Plain along the Adriatic. The classical situation is shown in Fig. 3. There are dune systems interposed between the sea and a series of lagoons, brackish ponds or cultivated lands obtained by draining old marshes (formerly extending from Ravenna up to Monfalcone). The dunes were originally colonized by oaks, ashes and hornbeams in cooler places; by relict patches of holly oaks (*Quercus ilex* L.) that are here at a northern limit of their distribution, in drier and hotter places. The original vegetation, however, was frequently replaced, already in

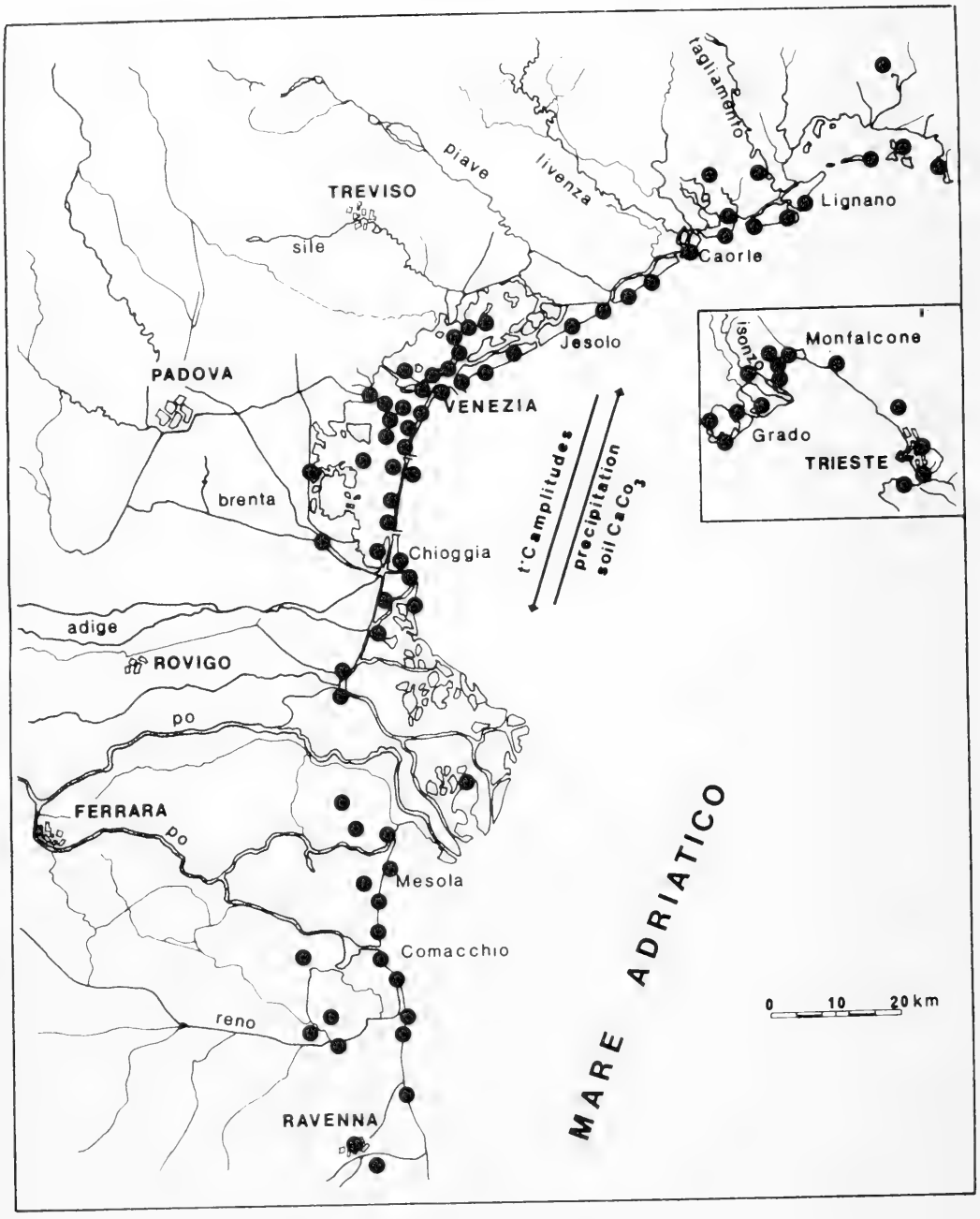

FIG. 1. Collecting stations, or groups of stations (1972–1982).

ancient times, with the more useful *Pinus pinea* L. and other pine trees.

Beside the marshes, such trees are replaced by willows and poplars, while common reeds (*Phragmites*) together with several Cyperaceae, Juncaceae and other "amphibious" grass-like plants, take the succession in the ecotone passing to aquatic vegetation.

C. nemoralis can inhabit the more sunny parts of those mesohygrophilous biotopes.

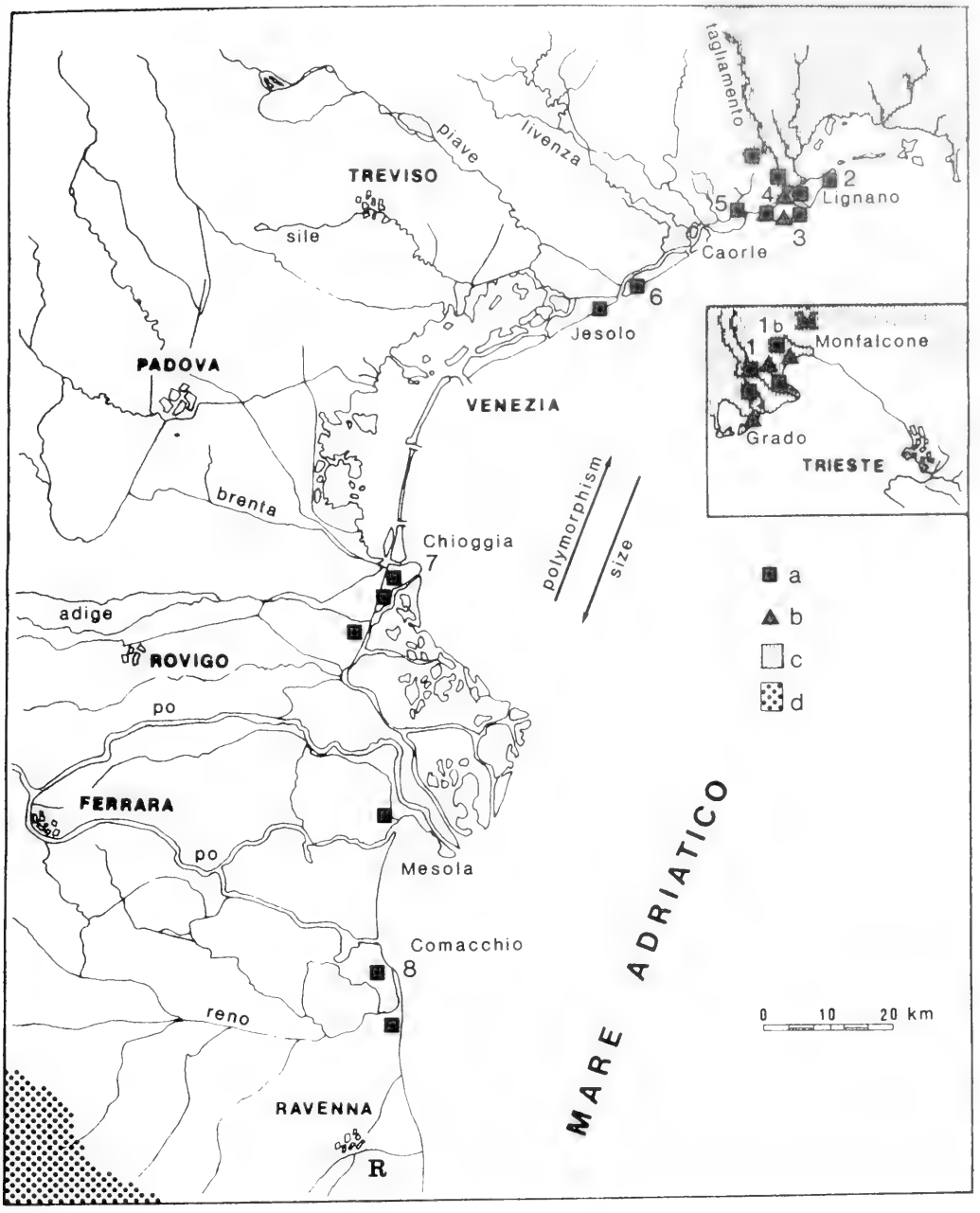

FIG. 2. a = *Cepaea nemoralis* (L.); b = *C. vindobonensis* (Fér.); c = potential area of *C. vindobonensis*, d = area of *Cepaea nemoralis apennina* (Stabile).

Intensive farming and human disturbance of dunes by an excessively heavy and poorly regulated touristic exploitation are nevertheless leading many small colonies to extinction, utterly damaging what remains of the natural landscape, with the rare exceptions of a few forestry reserves.

The north Adriatic coasts can be roughly divided, from a cepaeological point of view, into three main sectors:

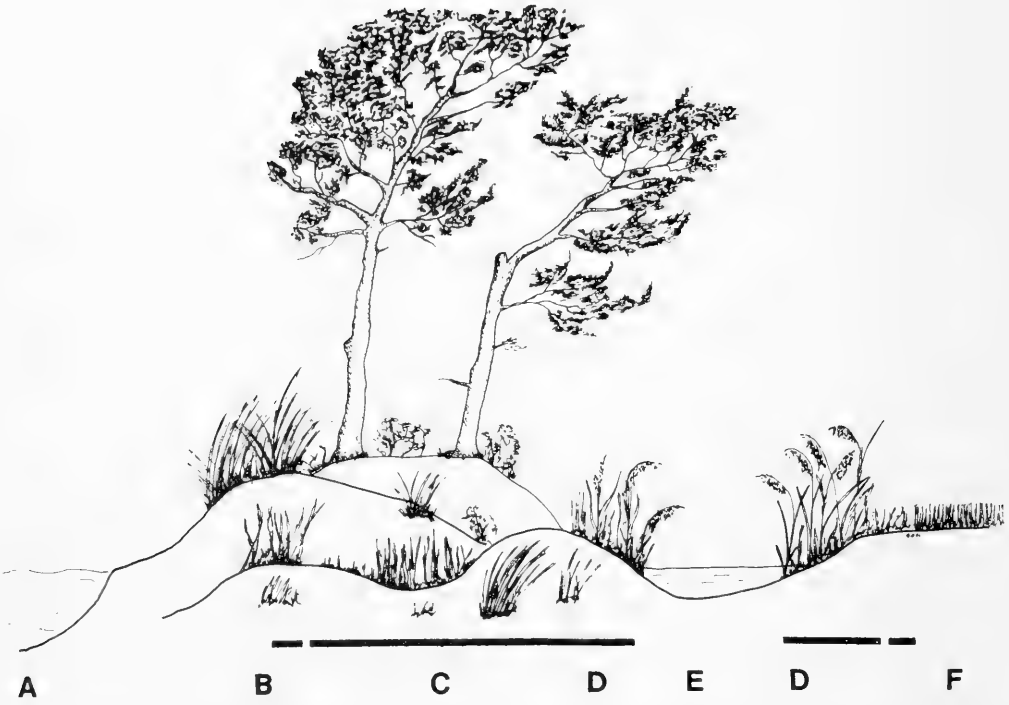

FIG. 3. A schematic transect of a littoral dune system. A = the sea; B = grass-fixed dunes with *Agropyrum* and *Ammophila*; C = littoral woods; D = *Phragmites* and other marsh grasses; E = littoral marshes and ponds; F = cultivated fields. The thick black line below the transect shows the potential range of *Cepaea* in the system.

1) The Friuli coast (Fig. 2, stations 1 to 5) from Monfalcone to the mouth of the River Tagliamento. This is the sector where some mixed populations of *C. nemoralis* and *C. vindobonensis* are found; the frequency of *C. nemoralis* is relatively high and its shell polymorphism is rich.

2) The territory around the Venice lagoon and the mouths of rivers which originally poured into the lagoon itself, and were successively diverted, to avoid its filling with sediments, some centuries ago (stations 6 and 7 of Fig. 2). It is the worst sector for *Cepaea,* due to the total alteration of the landscape, now crowded with industrial, agricultural and touristic establishments. *C. nemoralis* becomes in the Venice hinterland a ruderal species (Cesari & Orlandini, 1982).

3) The sector south of the Po delta (Station 8, Fig. 2) where *C. nemoralis* was never common, except at the moister and less thickly planted thresholds of some less disturbed pinewoods.

THE POLYMORPHISM

Tables 1 to 9 present data for the nine stations in which more than 50 snails were collected, belonging to one or both species of *Cepaea.* These data are summarized in Table 10 for *C. nemoralis* and in Table 11 for *C. vindobonensis.* For the latter, that has practically always a uniform ground colour (pale yellow to whitish) of the shell, a more detailed account is given of the band polymorphism.

A general discussion, however, is based not only on the data of the tables, but on other, smaller colonies too, so raising the total number of *C. nemoralis* to more than 1500, and of *C. vindobonensis* to 500. These figures should not be considered as particularly low, when compared with the discontinuous and infrequent presence of *Cepaea* in the districts investigated.

A few general trends can be recognized: 1. In *C. nemoralis,* five-banded shells, here all included in the morph class 12345 (the un-

TABLE 1. *C. nemoralis.* Sample No. 1-A. Ponte Primero.

Morphs	Yellow	Pink	T	%
00000	26	3	*29*	12.18
00300	4	5	*9*	3.78
00345	38	9	*47*	19.75
00045	43	3	*46*	19.33
02345	11	1	*12*	5.04
12345	65	20	*85*	35.71
Other	9	1	*10*	4.20
Total	196(82.35%)	42(17.65%)	*238*	
(Fused)	(5)	(6)	(11)	(5.26)
Dotted	80	22	102	48.80
Smudged	–	–	–	–

TABLE 2. *C. nemoralis.* Sample No. 1. Ponte Isonzo.

Morphs	Yellow	Pink	T	%
00000	66	8	*74*	21.76
00300	29	4	*33*	9.71
00345	33	16	*49*	14.41
00045	25	6	*31*	9.12
02345	20	6	*26*	7.65
12345	87	23	*110*	38.35
Other	16	1	*17*	5.00
Total	276(81.18%)	64(18.82%)	*340*	
(Fused)	(45)	(10)	(55)	(20.68)
Dotted	155	31	186	69.92
Smudged	–	1	1	0.38

TABLE 3. *C. nemoralis.* Sample No. 2. Lignano.

Morphs	Yellow	Pink	T	%
00000	2	–	*2*	4.44
00300	2	–	*2*	4.44
00345	–	1	*1*	2.22
00045	–	–	–	–
02345	1	1	*2*	4.44
12345	27	11	*38*	84.45
Other	–	–	–	–
Total	32(71.11%)	13(28.88%)	*45*	
(Fused)	(5)	(3)	(8)	(18.60)
Dotted	5	2	7	16.27
Smudged	–	–	–	–

TABLE 4. *C. nemoralis.* Sample No. 3. Bibione Faro.

Morphs	Yellow	Pink	T	%
00000	1	–	*1*	2.00
00300	3	2	*5*	10.00
00345	1	1	*2*	4.00
00045	–	–	–	–
02345	2	–	*2*	4.00
12345	18	22	*40*	80.00
Other	–	–	–	–
Total	25(50.00%)	25(50.00%)	*50*	
(Fused)	(5)	(2)	(7)	(14.28)
Dotted	4	3	7	14.28
Smudged	–	–	–	–

TABLE 5. *C. nemoralis.* Sample No. 4. Dunes of River Tagliamento.

Morphs	Yellow	Pink	Albino	T	%
00000	24	26	–	*50*	25.51
00300	1	6	–	*7*	3.57
00345	9	5	–	*14*	7.14
00045	–	–	–	–	–
02345	6	–	–	*6*	3.06
12345	48	59	2	*109*	55.61
Other	4	6	2(1.02%)	*10*	5.10
Total	92(46.94%)	102(52.04%)		*196*	
(Fused)	(11)	(14)	–	(25)	(17.12)
Dotted	3	–	–	3	2.05
Smudged					

TABLE 6. *C. nemoralis.* Sample No. 5. Terzo Bacino.

Morphs	Yellow	Pink	T	%
00000	10	8	*18*	8.33
00300	25	19	*44*	20.37
00345	–	1	*1*	0.46
00045	–	–	–	–
02345	1	1	*2*	0.93
12345	90	56	*146*	67.59
Other	3	2	*5*	2.31
Total	129(59.72%)	87(40.27%)	*216*	
(Fused)	(17)	(13)	(30)	(15.15)
Dotted	3	3	6	3.03
Smudged	–	–	–	–

TABLE 7. *C. nemoralis.* Sample No. 6. Eraclea Mare.

Morphs	Yellow	Pink	T	%
00000	3	–	*3*	2.50
00300	2	–	*2*	1.67
00345	5	–	*5*	4.17
00045	–	–	–	–
02345	1	1	*2*	1.67
12345	91	8	*99*	81.67
Other	9	–	*9*	8.33
Total	111(92.50%)	9(7.50%)	*120*	
(Fused)	(3)	–	(3)	(2.56)
Dotted	4	3	7	5.98
Smudged	–	–	–	–

TABLE 8. *C. nemoralis.* Sample No. 7. S. Anna di Chioggia.

Morphs	Yellow	Pink	T	%
00000	15	1	*16*	19.51
00300	27	7	*34*	41.46
00345	2	–	*2*	2.44
00045	–	–	–	–
02345	2	–	*2*	2.44
12345	24	2	*26*	31.71
Other	2	–	*2*	2.44
Total	72(87.80%)	10(12.19%)	*82*	
(Fused)	–	–	–	–
Dotted	1	1	2	3.03
Smudged	–	1	1	1.51

TABLE 9. *C. nemoralis.* Sample No. 8. Lido di Spina.

Morphs	Yellow	Pink	T	%
00000	12	2	*14*	10.80
00300	24	8	*32*	22.86
00345	16	5	*21*	15.00
00045	3	–	*3*	2.14
02345	2	3	*5*	3.57
12345	48	7	*55*	39.28
Other	8	2	*10*	7.14
Total	113(80.71%)	27(19.28%)	*140*	
(Fused)	(4)	(4)	(8)	(6.34)
Dotted	20	7	27	21.42
Smudged	1	2	3	2.38

TABLE 10. *C. nemoralis.* Totals.

Morphs	Yellow	Pink	Albino	T	%
00000	159	48	–	*207*	14.51
00300	117	51	–	*168*	11.77
00345	104	38	–	*142*	9.95
00045	71	9	–	*80*	5.61
02345	46	13	–	*59*	4.13
12345	498	208	2	*708*	49.61
Other	51	12	–	*63*	4.41
Total	1046(73.30%)	379(26.56%)	2(0.14%)	*1427*	
(Fused)	(95)	(52)	–	(147)	(12.04)
Dotted	275	72	–	347	28.44
Smudged	1	4	–	5	0.40

TABLE 11. *C. vindobonensis.* Samples.

Morphs	1-A	2	3	4	Total	%
12345	*5*	*26*	*99**	*56*	*186*	56.36
1(23)45	4	35	30	7	*76*	23.63
1[23]45	1	10	29	3	*43*	13.03
1:345	–	–	8	1	*9*	2.73
10345	–	1	2	–	*3*	0.91
::345	–	–	–	1	*1*	0.30
123 45	–	–	–	1	*1*	0.30
[1(23)]45	–	–	2	1	*3*	0.91
[123]45	–	–	1	1	*2*	0.60
[12]345	–	–	1	–	*1*	0.30
(123)45	–	1	–	1	*2*	0.60
[(12)3]45	–	1	–	1	*2*	0.60
(12)345	–	–	–	1	*1*	0.30
Total	10	74	173	74	330	

*albino = 1; smudged = 3.

common fusions being considered apart in the statistics) represent about 50% of the total. This high frequency of complete banding may be related to the original local landscape (mainly of coastal woods). 2. The high frequency of interrupted (= dotted) bands in Friuli are in disagreement with the hypothesis connecting dotted banding with particularly dry microclimates for *C. nemoralis.* On the contrary, pale bands in *C. vindobonensis* are only found in the driest station (Station 3, near the Bibione lighthouse). Pale (= smudged) banding is everywhere rare in *C. nemoralis.* 3. The relatively low percent of pinks (with the important exception of the two stations near Bibione) is not an uncommon feature in northeastern Italy, where colonies entirely formed by yellows are frequently found. Such a situation leads Testa (1958) to the evidently incorrect statement that a longitudinal gradient of pink frequencies in Northern Italy drives this character to complete extinction east of Lake Como (central Lombardy). 4. Albino shells are extremely rare in comparison with other Italian districts (Tagliani, 1942; Sacchi, 1980). 5. No white lip was found in adult shells, except of course in the two albinos of Station 4. 6. The highest degree of polymorphism is found among the Friuli colonies. As for visual predation, there are but a few shells of *C. vindobonensis* eaten by rats at Station 3, near the mouth of Tagliamento, and a number of shells broken by birds (possibly by pheasants) at Station 8, that is now a part of a small forestry reserve. One should moreover take into account the appreciation as food of *Cepaea* by Friulian and Venetian farmers, together with the more sophisticated *Helix pomatia* L., *H. lucorum* L., *H. cincta* Müll. and *Cryptomphalus aspersus* (Müll.) often found in the same stations. The same is true elsewhere in Northern Italy (Sacchi & Valli, 1975).

The average shell size of *C. nemoralis* slightly increases towards the south. The shell is about 20–22 mm (breadth) × 16–18 mm (height) in Friuli, where *C. vindobonensis* is larger and higher (22–23 × 19–20 mm). The shell of *C. nemoralis* measures 23–24 mm × 18–19 mm south of river Po (Station 8: data in preparation).

DISCUSSION

This research is a part of a long-term study whose aim is to find bionomic and ecological

effects of the main environmental factors, by the analysis of occurrences, biotic cycles and, whenever possible (as is the case here) polymorphic variations of the larger land molluscs along the north Adriatic coasts of Italy.

Preliminary discussion is nevertheless possible. The northeastern corner of the great alluvial plain watered by the River Po and Venetian rivers shows, on its maritime border too, some characters of subatlanticism together with some alpine features. The former is given in *C. nemoralis* by a richer polymorphism, high frequencies of pinks in several sites, as well as by more frequent, less discontinuous and larger colonies. This means more convenient environmental conditions for mid-European snails, due to higher annual rainfalls, cooler summers and relatively small thermic amplitudes, i.e. a suboceanic climate (Sacchi, 1977, 1978).

The latter are emphasized by the occurrence on littoral dunes of *C. vindobonensis,* that in Italy usually behaves as a mountain species, coming down to the sea along the rivers Tagliamento and Isonzo, which supply a natural and usual way of invasion into the Friuli plain for Alpine populations—of plants, animals and men (Sacchi, 1983).

South of the Po delta, that is a poor territory for *Cepaea* with its recent, frequently flooded, and yet ruthlessy harrowed lands, *C. nemoralis* shows characters slowly leading to the geographic race *apennina* Stabile, endemic to the Italic peninsular territory. The splitting of *C. nemoralis nemoralis* from *C. n. apennina* does not seem here to be so sharply traced as it is elsewhere, e.g. in Southern Lombardy (Sacchi & Valli, 1975). South-padanian colonies of *C. nemoralis,* however, are frequently small, isolated, looking like mere survivors, possibly in relation not only with sands poorer in lime (10–15% $CaCO_3$ as compared with 70–75% in Friuli dunes) (Violani, 1977) but also with harsher climatic conditions. South of the delta, summer becomes hotter, drier and longer, already announcing truly Mediterranean conditions. Individual sizes of *C. nemoralis* become larger, though not so large as in populations from the north-western Apenninian slopes (Sacchi & Valli, 1975).

The variation of one species then fits into the general biogeographic frame admitted for the maritime border of the Po Plain. The large delta constitutes a biogeographic limit (Sacchi, 1978) between a northern sector, with a reduced mediterraneism (the so-called "north Adriatic gap," excluding a number of thermophilous and xerobic species, but including some Atlantic-Adriatic stocks both among animals and plants: Sacchi (1977) and a southern sector, gradually merging into fully Mediterranean biochores.

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REFERENCES CITED

CESARI, P. & ORLANDINI, M., 1982, Nuovi reperti di *Cepaea nemoralis* (L.) nel Veneto ed in Emilia-Romagna. *Atti Società Veneziana di Scienze Naturali,* 7: 125–226.

PIERSANTI, C., 1926, *Intorno alla variabilità della conchiglia dell'* Helix nemoralis L. Tip. Edit. Fiori, Jesi, 35 p.

SACCHI, C. F., 1977, La "lacune nord-adriatique" et son influence sur l'écologie des Gastéropodes dunicoles. Prémisses méthodologiques. *Atti della Società Italiana di Scienze Naturali,* 118: 213–225.

SACCHI, C. F., 1978, Il delta del Po come elemento disgiuntore nell'ecologia delle spiagge adriatiche. *Bollettino del Museo Civico di Storia Naturale di Venezia,* 29 Suppl.: 43–78.

SACCHI, C. F., 1980, Contributo alla demoecologia di *Cepaea nemoralis apennina* Stabile. *Natura, Milano,* 71: 53–68.

SACCHI, C. F., 1983, Il Nord Adriatico: crocevia di faune, intreccio di popoli. *Atti del Museo Civico di Storia Naturale di Trieste,* 35: 39–64.

SACCHI, C. F. & VALLI, G., 1975, Recherches sur l'écologie des populations naturelles de *Cepaea nemoralis* (L.) en Lombardie méridionale. *Archives de Zoologie Expérimentale et Générale,* 116: 549–578.

TAGLIANI, G., 1942, Contributo statistico biometrico allo studio delle conchiglie di *Cepaea nemoralis* var. *apennina. Bollettino di Zoologia,* 13: 159–171.

TESTA, D., unpublished (1958), Polimorfismo in popolazioni naturali di *Cepaea nemoralis* (L.). Tesi di Laurea Sc. biol. Univ. Milano, 76 p.

VIOLANI, C., 1978, Per una conoscenza delle spiagge sabbiose dell'alto Adriatico. Dati pedologici a nord ed a sud del Delta del Po. *Bollettino del Museo Civico di Storia Naturale di Venezia,* 29 Suppl.: 87–110.

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POLYMORPHISM OF *CEPAEA NEMORALIS* (GASTROPODA, HELICIDAE) IN THE SPANISH OCCIDENTAL PYRENEES

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ABSTRACT

Samples of *Cepaea nemoralis* have been taken in four Spanish Pyrenean valleys (Caldarés, Canfranc, Esca and Salazar) in order to investigate the factors that may influence the phenotypic composition of those populations.

Two different approaches have been explored, the relationships between morph frequencies and climate, topography and vegetational zones and between these frequencies and the nature of the biotopes (open or shaded, tall or short herbage, etc.).

Rainfall does not seem to be an important factor in determining morph frequencies as yellow shells increase with decreasing rainfall in a longitudinal trend but not in a latitudinal trend within the valleys.

The two longest and most similar valleys, Canfranc and Esca, show a similar pattern of distribution of yellow shells with the highest frequencies of this morph in similar wide alluvial plains of the valleys. It is suggested that the marked seasonal oscillations in temperature and rainfall of these zones may account for this colour pattern. However, variation of colour patterns among the same vegetation zones of the four valleys can not be solely explained on a climatic basis, and the same can be concluded about banding.

A factor analysis was carried out, showing that morph frequencies can be more consistently related to biotopes. Yellow unbanded snails are most frequent in open biotopes and pink banded in shaded ones, with intermediate frequencies of both in intermediate biotopes. Such a relation is thought to be the result of differences between the two kinds of shells in the strategies of metabolic response to temperature amplitudes of the biotopes. In this sense it must be pointed out that 1) yellow increases in frequency when shade and density of vegetation decrease, 2) high frequencies of fused bands are only found in shaded biotopes, and 3) hyalozonate bands are frequent in open biotopes of the wider part of the Canfranc and Esca Valleys, where unbanded decreases. It is suggested that hyalozonate might act as a counterbalance for the relative decrease of unbanded in such biotopes.

Other characters (white lip, punctate bands and variants of the number of bands) show an irregular distribution independent of climate, vegetational zones, topography or biotope, which may be taken as an indication of a selectively neutral value for these rare alleles or of the existence of other cryptic factors.

Key words: *Cepaea nemoralis*; polymorphism; Spanish Pyrenees.

INTRODUCTION

Pyrenean populations of the land snail *Cepaea nemoralis* (L.) show great variations in morph frequencies within and among valleys. Several authors have made attempts to find the causes of this complex and heterogeneous pattern (Lamotte, 1951, 1968a,b; Arnold, 1968 and Cameron *et al.*, 1973 in the Central Pyrenees and Jones & Irving, 1975 in the Eastern Pyrenees).

The work of Arnold (1968, 1969) in the Garona and Segre-Valira Valleys shows that changes in morph frequencies are associated, in a general fashion, with variations in altitude, aspect and orientation of the valleys. This author suggested that these changes might be due to climatic selection. However, later investigations led Cameron *et al.* (1973) and Jones & Irving (1975) to conclude that the different trends in morph frequency variation in the Southern Pyrenean valley systems do not fall within the scope of a simple climatic interpretation. Thus, other hypotheses about selective and non-selective factors (see review in Jones *et al.*, 1977) must be considered.

We have explored two different approaches in order to inquire about the factors that may influence the morph composition of the *C.*

nemoralis populations of the Caldarés, Canfranc, Esca and Salazar Valleys (Central-Occidental Pyrenees). In the first one, the relationships between climate, vegetational zonation and topography along the altitudinal gradient of the valleys and morph frequencies are considered, whereas in the second, we consider the relationships between the nature of the biotopes and colony composition.

In the first case, we hope to see how changing conditions of the environmental factors above mentioned (in altitude along the valleys and in longitude in a east-westward trend) are associated with change in morph frequencies, leading to some definite pattern of variation. In the second case, we hope to see if some pattern of variation not explained by more or less general environmental factors, may be better related to the background on which the colonies are found.

MATERIAL AND METHODS

The area

Valleys surveyed are, from east to west, Caldarés, Canfranc, Esca and Salazar. They are shown in Fig. 1 and are in the South Occidental Pyrenees. Soler-Sampere & Puigdefábregas (1972) have given a detailed account of the geology in this region. Climatic data were obtained from Dr. J. Puigdefábregas (personal communication) and the Servicio Meteorológico Nacional.

The vegetational zonation considered is, according to Montserrat (1971):

A. Sub-mediterranean zone, characterised by the dominance of box (*Buxus sempervirens* L.) frequently associated with *Genista scorpius* D.C. in the drier places. This kind of vegetation corresponds to a moderately rainy

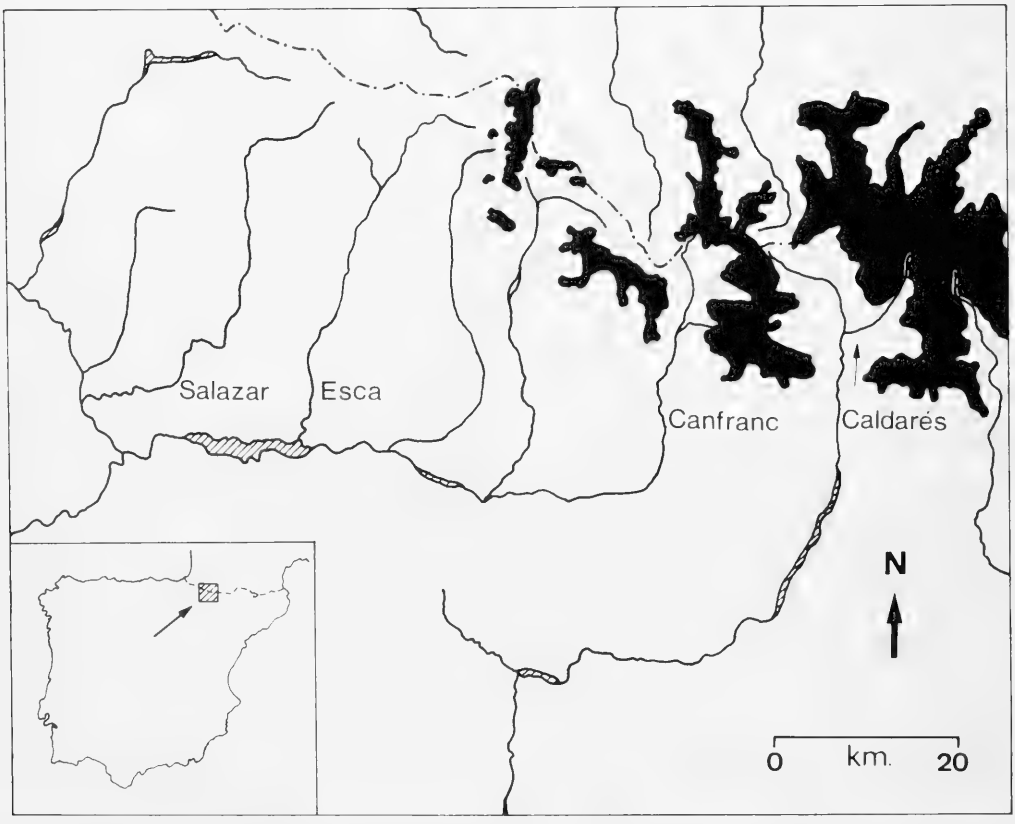

FIG. 1. Map showing the location of the Caldarés, Canfranc, Esca and Salazar Valleys (South Occidental Pyrenees). Black areas represent land above 2000 m.

climate (600–1000 mm) but with an irregular distribution of the rain, with hot summers and cold winters. In the drier and more windy areas box is mixed with small holm oak.

The transition to the montane zone is gradual, plants more exigent in humidity and more resistant to cold appearing progressively.

B. Dry montane zone, corresponding to the colder areas with frost in May–June and with not enough dampness for the beech-fir forest. The climax is pine woods with box, somewhat degraded by clearance, thus at present mainly box.

C. Damp montane zone, which is very reduced in this region. We distinguish three different kinds:

1) Elm groves at the ravinesides and riversides with damp soils. They are composed of mixed vegetation of *Populus* spp., *Ulmus* spp., *Salix* spp., etc. and many submediterranean herbaceous species.

2) Wet areas poorly covered, abundant in *Cirsium monspessulanum* All.

3) Fir wood with beech. At present this climax vegetation has been cleared and tall box trees cover wide areas forming small woods. This kind of vegetation is placed in areas open to oceanic influence. We have found it only at the head of the Esca Valley.

D. Sub-alpine zone. It differs substantially in soil nature, climate and vegetation in each valley (see below).

The Caldarés Valley

It is the easternmost valley of the four studied, placed in the axis of the Pyrenees. The valley was sampled from 1200 m to 1600 m. The soil is clayey slate mixed with sandstone and limestone in the lower zone, while granites predominate above 1400 m. Montane vegetation is found in the valley except for the uppermost part, which is a sub-alpine zone with *Pinus uncinata* Mill. ex Mirb. and *Rhododendron.* Only one sample was taken from this zone.

The Canfranc Valley

This valley is formed by the Aragón river. *C. nemoralis* was sampled from 750 m to 1480 m. The lower valley is made up of continental sediments and in the middle the valley is open in a wide alluvial plain with submediterranean vegetation somewhat degraded, being very rich in *Buxus sempervirens.* Above 1100 m the river flows between limestone mountains and the valley becomes narrower. This is a dry montane zone with *Pinus silvestris* L. and box very abundant. The two uppermost samples were taken from a sub-alpine steppe vegetation with box (there is no typical sub-alpine vegetation). The lower of these two localities (A18) has also other species such as *Juniperus communis* L., *Androsace villosa* L., *Saponaria caespitosa* D.C. and *Thymelaea tinctoria nivalis* (Ramond) Mont., suggesting the existence of climatic differences between the two sites perhaps affecting their phenotypic composition.

The Esca Valley

Samples were taken from 450 m to 1750 m. Below 600 m the valley is open and dry. From 600 m to 850 m it goes through a succession of limestone gorges and wider alluvial areas. This region has a submediterranean character somewhat degraded by grazing, constituted by box, oak scrub and/or furze. Due to limestone mountains the tributaries flow in the east-west direction. Above this altitude the river flows through a broad glaciated valley (Belagua) and is under some mild oceanic influence. The montane climax vegetation of this zone, pure beech forest with fir, is degraded and box abundant. The uppermost two samples were taken from pine groves (*Pinus uncinata*) with herbaceous plants of *Festuca scoparia* Kern. and common juniper of the sub-alpine level, the substrate of which is constituted by karst limestones.

The Salazar Valley

The influence of the east-west chain of limestone mountains is less important in this valley where flysch dominates, and the valley is open with wide alluvial plains. The upper four samples were taken (above 650 m) from the montane zone. In the sub-mediterranean zone, there are clear differences in morph frequencies between valley bottom samples and hillside samples, taken from a more mediterranean vegetation with calcareous soil.

Sampling methods

Samples of *C. nemoralis* were collected between 1975 and 1977 in all the vegetational zones existing in each valley by searching

areas smaller than the panmictic unit for the species (Lamotte, 1951). All live adult specimens found were collected, but only samples containing more than 15 snails have been considered. Shells were scored for colour and banding according to the criteria given by Cain & Sheppard (1950, 1954). Each sample is referred to here as a colony.

Sampling showed no significant variations in morph frequency within the colonies during the period of our study, so that the results are considered all together in Tables 8, 9,10 and 11.

Characteristics of the biotope and vegetation were noted for each locality.

Statistical methods

To summarize and quantify the overall pattern of geographic variation of the characters a principal components factor analysis (PCA) of the phenotypic frequencies was performed using the Lebart & Fénelon (1975) ACOMPP programme.

The selected characters for this analysis are: shell colour (yellow, pink, brown), banding (unbanded, any banded including hyalozonate), lip colour (white lip, dark lip) and coloration of the bands (hyalozonate bands, normal pigmented bands).

To evaluate associations between pairs of characters, contingency χ^2 tables (2 × 2) were calculated.

RESULTS

General polymorphic traits in the area under study

Yellow is the most frequent colour of shell in all the valleys. Its mean frequency is above 65% while brown is rare and irregularly distributed.

Concerning the bands, unbanded (00000) and five-banded (12345) are the commonest banding morphs in the four valleys. Among the banded shells, we have found 15 of the 31 possible combinations with one to five bands (Table 1). Most frequent modifiers are those leading to 00300 and 00345 shells, but they have an irregular distribution, being common only locally. The other minor variations are rare or absent. Esca is the valley where the number of modifiers of banding is highest.

Lip colour is also polymorphic in nearly all the colonies, and white lip is frequent mainly in the Salazar and Canfranc valleys, but it shows local variations.

Hyalozonate and punctate bands are present in all the valleys with higher frequencies than those found in the Oriental Pyrenean valleys (Cameron *et al.,* 1973; Arnold, 1968); they are widely distributed with important local variations. Hyalozonate is more frequent in the Canfranc and Esca Valleys, while the frequency of punctate bands decreases from east (Caldarés) to west (Salazar).

The variation of some characters is not independent from that of others. For example, white lip shows a significant excess among yellow shells with respect to pink shells (Table 2), and this pattern is repeated in the four valleys. The association of white lip with unbanded is highly significant as well (Table 3). The populations of the four valleys show a clear statistical association between yellow and unbanded (and therefore pink and

TABLE 1. Frequencies of the different banding morphs in the four valleys surveyed.

Banding variants	Caldarés	Canfranc	Esca	Salazar
00000	63.91	51.55	50.95	79.40
00300	5.55	8.48	9.64	3.64
00005	—	—	—	0.27
10300	—	0.16	—	—
00340	—	—	0.38	—
00305	0.08	—	0.12	—
00045	—	—	0.11	0.31
10305	—	0.11	0.01	—
02340	—	—	0.49	—
00345	2.91	1.55	4.10	5.88
12340	—	—	0.07	—
12305	—	—	—	0.15
12045	—	—	0.49	—
10345	3.01	5.21	1.95	1.43
02345	—	0.19	1.28	0.17
12345	24.52	32.58	30.74	8.98

TABLE 2. Distribution of white lip among yellow and pink shells in four valleys of the Occidental Spanish Pyrenees.

Valley	Frequency of white-lip		P
	Yellow	Pink	
Salazar	0.38	0.21	<0.001
Esca	0.30	0.18	<0.001
Canfranc	0.37	0.28	<0.02
Caldarés	0.16	0.06	<0.001

TABLE 3. Distribution of white lip among unbanded and banded shells in four valleys of the Occidental Spanish Pyrenees.

Valley	Frequency of white-lip		P
	Unbanded	Banded	
Salazar	0.37	0.25	<0.02
Esca	0.33	0.17	<0.001
Canfranc	0.53	0.12	<0.001
Caldarés	0.17	0.06	<0.001

TABLE 4. Distribution of unbanded among yellow and pink shells in four valleys of the Occidental Spanish Pyrenees.

Valley	Frequency of unbanded		P
	Yellow	Pink	
Salazar	0.85	0.58	<0.001
Esca	0.66	0.40	<0.001
Canfranc	0.59	0.40	<0.001
Caldarés	0.76	0.35	<0.001

banded (Table 4). Such disequilibrium holds both in shaded and in open biotopes (see below and Table 7). Mid-banded, unlinked with colour and banding (Lamotte, 1954; Cain & Sheppard, 1957; Cain *et al.*, 1960), shows

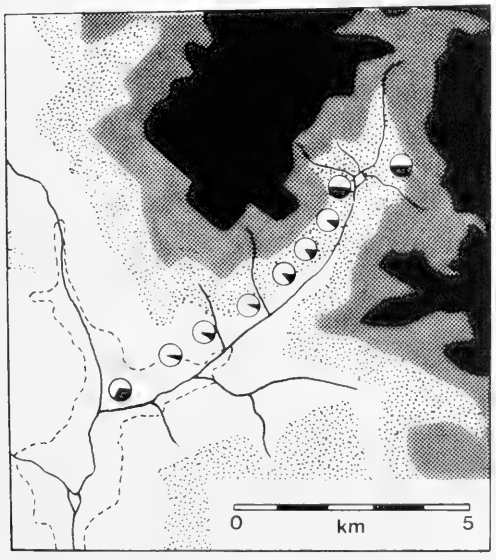

FIG. 2. Frequency of yellow (white), pink (black) and brown shells (dotted sector) in the Caldarés Valley. Contours at 1200 m (dashed line), 1600 m, 2000 m and 2400 m.

TABLE 5. Distribution of mid-banded (00300) among yellow and pink shells in four valleys of the Occidental Spanish Pyrenees.

Valley	Frequency of 00300		P
	Yellow	Pink	
Salazar	0.18	0.23	n.s.
Esca	0.16	0.19	n.s.
Canfranc	0.16	0.05	<0.001
Caldarés	0.16	0.24	n.s.

the same frequencies among yellow and pink shells except for the Canfranc Valley where it is in excess among yellow (Table 5).

Variation within the valleys

The Caldarés Valley

Yellow, pink and brown shells, as well as banded and unbanded are present in the valley from 1200 m to 1600 m (Table 8). Above this altitude the frequency of yellow drops to 50% (Fig. 2) while the frequency of brown shells increases to 12.5% in the upper population inhabiting the sub-alpine zone. This sample exhibits the highest frequency (near fixation) of banded shells although unbanded shells are the most frequent in the valley as a whole (Fig. 3). Frequency distributions of yellow and unbanded morphs are similar.

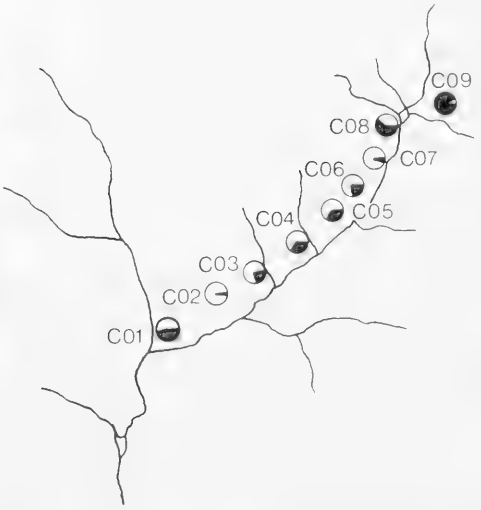

FIG. 3. Frequencies of unbanded (white sector) in the Caldarés samples.

White-lipped shells are only in small numbers. Modifiers of the band system are rare, and punctate, irregularly distributed, reaches high frequencies.

The Canfranc Valley

All the colonies are polymorphic for the three major loci (shell colour, banding and lip colour). Pink shells tend to be more frequent at lower and higher altitudes while yellow characterises the intermediate zone of the valley from 900 m to 1200 m (Table 9). The samples from this zone are very similar in frequency of yellow (Fig. 4). Brown shells are less common in this valley, being only present in nine samples; their frequency is very low in the intermediate zone.

Fig. 5 shows the percentages of unbanded shells. The frequency of this morph tends to increase with altitude. In the intermediate zone there are moderate frequencies of unbanded shells. Mid-banded (00300) modifier and hyalozonate bands that are well represented in the valley reach their highest frequencies here. Mid-banded and punctate bands show local variations (Table 9).

The frequency of white lip varies from 0.5 to 77%. In spite of important local variations, more marked among yellow shells than among unbanded shells, it tends to increase its frequency with altitude.

The Esca Valley

Details of the scores, biotopes and altitudes of each sample are given in Table 10.

Fig. 6 illustrates the distribution of frequencies of yellow and pink shells. There is a lower zone (below 625 m) where pink reaches moderate frequencies. This morph is also relatively common above 800 m. The

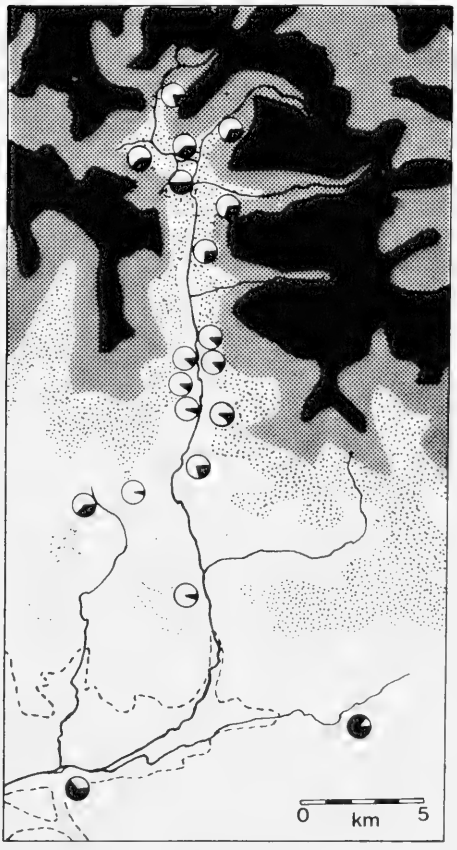

FIG. 4. Frequency of yellow (white) and pink (black sector) in the Canfranc Valley. Contours at 800 m (dashed line), 1200 m, 1600 m and 2000 m.

FIG. 5. Frequencies of unbanded (white sector) in the Canfranc samples.

highest frequencies of yellow are found in the intermediate samples (625 m to 800 m).

The frequency distribution of brown is similar to that of pink, being more abundant in the lower and higher parts of the valley, though it is absent in many samples. This morph may be locally common (i.e. in the sample E13 it reaches 41.6%).

Unbanded shells do not show changes in frequency that might be associated with altitude (Fig. 7). This valley has the highest proportion of shells with less than five bands (00300, 9.64%; 00345, 4.1%), distributed with important local variations. Punctate bands are frequent in the lower and upper parts of the valley and hyalozonate bands in the intermediate zone.

Local variations in the frequency of white lip are more marked than in the other valleys.

The Salazar Valley

The frequency of yellow shells and unbanded shells is remarkably high throughout this valley (Table 11, Figs. 6 and 7). Both alleles are fixed in three hillside samples from the lower Salazar. The valley has the highest mean frequency of unbanded shells. In opposition to the other valleys the frequency of 00345 is higher than that of 00300. These banding modifiers are common in some localities. Only two samples have brown shells. No punctate bands were found.

Variation with biotope

Summarizing and quantifying the variation of morph frequencies was carried out through a principal components analysis. Factor loading for phenotypic frequencies of nine morphs representing the main four polymorphic loci in these valleys are shown in Table 6, and factor scores for each of the 80 localities sampled in Fig. 8. Factor I has its strongest loading for banding and colour but is also associated with lip colour. It separates localities into three groups: a) A first group that includes the most shaded biotopes of the C-1 vegetational zone, and the sub-alpine zone (D) when it is made of *Pinus uncinata* woods. Samples of this group have the highest frequencies of pink and banded shells. b) The second group includes the samples from the most open and dry vegetation corresponding to the A, B and C-2 vegetational zones. These samples are characterised by high frequencies of yellow

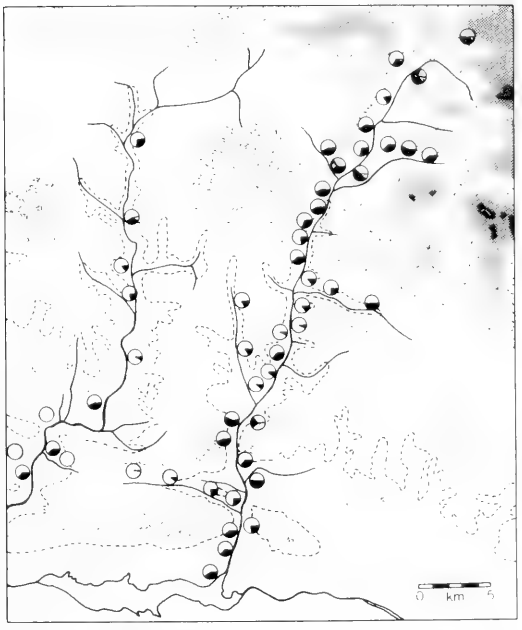

FIG. 6. Frequency of yellow (white), pink (black) and brown shells (dotted sector) in the Salazar (left) and Esca (right) Valleys. Contours as in Fig. 4.

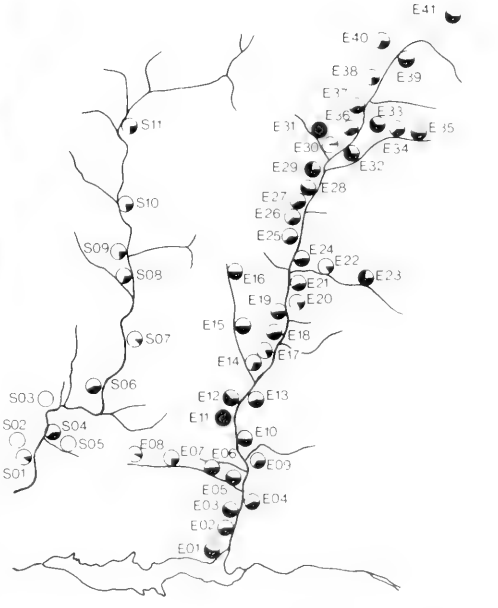

FIG. 7. Frequencies of unbanded (white sector) in the Salazar (left) and Esca (right) samples.

TABLE 6. Factor loading for phenotypic frequencies of nine morphs of *Cepaea nemoralis* in four valleys of the Occidental Spanish Pyrenees.

Morph	Factor 1	Factor 2	Factor 3
Yellow	−0.858	0.006	−0.448
Pink	0.814	−0.027	0.435
Brown	0.455	0.062	0.208
White lip	−0.745	−0.191	0.624
Dark lip	0.746	0.189	−0.623
Unbanded	−0.825	0.427	−0.020
Banded	0.826	−0.427	0.022
Normal pigmented bands	0.223	0.943	0.135
Hyalozonate bands	−0.223	−0.943	−0.136

and unbanded shells. c) A group of samples with mean frequencies of these two characters is found in the most heterogeneous biotopes (C-3 vegetational zone, localities more exposed to human activity or cleared woods of C-1). This axis represents 46% of the phenotypic variation, averaged over the nine morphs.

Factor II represents an average of 25% of the variation in phenotypic frequencies. The highest loading on this factor is hyalozonate bands which separates those localities of the A zone situated in the parts where the intermediate zone of the Esca and Canfranc Valleys becomes wider, from those of the narrower and lower parts of these valleys. Factor III which has heavy loading on lip colour represents an average of 14% of the variation. White lip is positively correlated with unbanded and yellow morphs. Thus, this Factor merely introduces some dispersion into the groups already determined by Factor I. None of these three Factors appear to be correlated with altitude (i.e. correlation coefficient between Factor I, which represents the variation of shell colour and banding, and altitude is $r = 0.0238$, n.s.).

The frequency of unbanded increases in open biotopes and is significantly higher among yellow shells than among pink shells, both in shaded and open biotopes (Table 7). It is necessary to except some samples situated in the wider part of the Canfranc and Esca Valleys (the other two valleys have not such parts), in which a) unbanded decreases and seems to be distributed almost equally between yellow and pink shells, and b) hyalozonate increases in frequency and is equally distributed between yellow and pink shells (freq. 0.075 and 0.064, respectively).

TABLE 7. Distribution of unbanded among yellow and pink shells in different biotopes of the Occidental Spanish Pyrenees.

Biotope	Frequency of unbanded		P
	Yellow	Pink	
Shaded	0.34	0.12	<0.001
Open	0.82	0.58	<0.001
Open (wider part)	0.54	0.49	n.s.

DISCUSSION

A. Variation of morph frequencies in relation to changing environmental factors along the valleys

Differentiation along (and among) valleys would not be surprising because of the differences in the altitudinal ranges of vegetation zones and climate existing in each of them. The oceanic influence coming from the northwest is marked in Salazar, at the higher and lower Esca and at the head of Canfranc, whereas Caldarés, the most eastern valley, escapes from it. Thus, from west to east there is a decrease in rainfall that might be related to the increase in the overall frequency of yellow shells as suggested by Guerrucci-Henrion (1966) for Brittany. However, when such a pattern of variation of rainfall is considered along the altitudinal gradient of the valleys, this explanation does not stand up, as yellow shells do not decrease with altitude in relation to the increasing rainfall. Here we have an example of the frequent contradictions found about the distribution of morph frequencies of *Cepaea* in the Pyrenees and other areas. Indeed, Harvey (1971) could not find the same relationship for Southwestern France and Northern Spain as Guerrucci-Henrion found for Brittany.

If one considers the effects of temperature and rainfall in relation to seasonal oscillations, the explanation of shell colour pattern appears to be more satisfactory. The two longest and ecologically most similar valleys, Canfranc and Esca, show a similar pattern of variation in the frequencies of yellow shells. The highest frequencies of this morph are found in the intermediate part of these valleys at altitudes that, being different in each valley, really correspond in both cases to the same wide alluvial plain of the sub-mediterranean

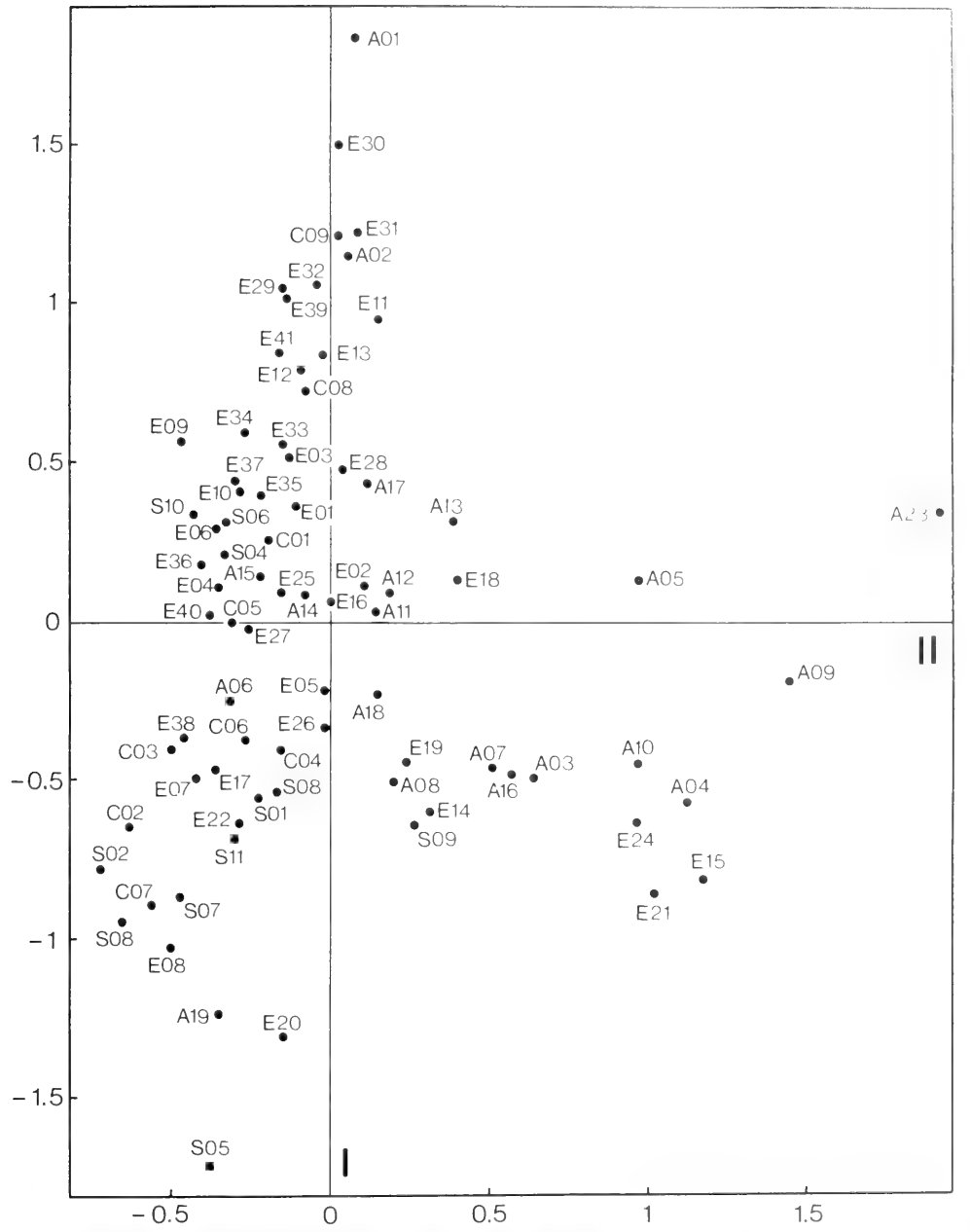

FIG. 8. Distribution of colonies with respect to the first two principal components. Axes I and II have each 4.2 and 2.2 as proper values and represent 46% and 25% of the variance.

zone close to the montane zone. In these areas, both the percentage of precipitation during May–June and the daily thermic oscillations of the summer are higher than in other parts of the valleys. These factors may account for the observed high frequency of yellow shells, if, as it has been suggested, this morph is more resistant to extreme climatic conditions (Arnold, 1968, 1971; Carter, 1968; Cameron, 1969; Richardson, 1974 and oth-

TABLE 8. Results of sampling *C. nemoralis* in the Caldarés Valley. "Others" include all the banding variants different from 00300 and 12345. wl = white lip; dl = dark lip. Y = yellow, P = pink, 5b = 12345, 1b = 00300, 3b = 00345. Numbers in parenthesis represents punctate bands. Abbreviations of vegetational zones as in material and methods. S = shaded biotopes, 0 = open biotopes, OW = open biotopes from the wider part of the valley, I = intermediate biotopes, D = disturbed biotopes.

					Yellow								Pink								Brown					
					00000		00300		12345		Others		00000		00300		12345		Others		00000		Banded			
Sample Nº.	U T M Coordinate	Altitude (m.)	Veget. zone	Biotope type	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	Hyalozonate	Total
C01	30TYN211347	1200	C-1	I	8	28	1	1	-	14(1)	1	5	1	9	-	4	-	10	-	2(1)	-	2	-	2	1Y5b, 1Y10345	90
C02	30TYN217340	1250	A	O	5	65	-	1	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	1Y5b	74
C03	30TYN233346	1320	B	O	21	73	-	4	-	8(2)	1	7(1)	-	7	1	2	-	3	-	-	-	1	-	2		130
C04	30TYN241351	1360	B	O	8	37	1(1)	3	2(2)	10(4)	1	4(1)	-	2	-	1(1)	-	1	-	-	-	-	-	-	1Y1b, 2Y3b	73
C05	30TYN248358	1450	B	I	-	24	-	1	-	6	-	3(1)	-	1	-	-	-	2	-	-	-	-	-	2(1)	1Y3b,	40
C06	30TYN256368	1540	C-2	O	14	102	-	2	-	15	-	5	-	11	-	-	-	5	-	3	-	5	-	-	5Y5b, 2Y1b, 1P1b	170
C07	30TYN257370	1600	C-2	O	16	24	-	-	-	2	-	-	1	4	-	-	-	-	-	-	-	-	-	-		47
C08	30TYN260375	1659	B	I	15	34	-	10(5)	3(2)	20(7)	-	2	4	17	1(1)	15(2)	2	38(9)	-	8(1)	-	2	-	8(1)	1P5b, 2P1b	182
C09	30TYN267385	1780	D	S	-	2	-	-	2	10	-	2	-	-	-	2	-	10	-	-	-	-	-	4		32

TABLE 9. Results of sampling *C. nemoralis* in the Canfranc Valley. Abbreviations as in Table 8.

Sample Nº.	U T M Coordinate	Altitude (m.)	Veget. zone	Biotope type	Yellow								Pink								Brown					
					00000		00300		12345		Others		00000		00300		12345		Others		00000		Banded			
					wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	Hyalozonate	Total
A01	30TYN076149	825	C-1	S	-	5	-	-	1	59	-	-	1	7	-	-	-	367	-	-	-	2	-	4	2Y5b, 2P5b	450
A02	30TXN965145	750	C-1	S	3	14	-	1	4(2)	26(2)	1	3	2	12	-	3	2(1)	68(2)	-	4	-	2	-	5	3Y5b	153
A03	30TXN012223	900	A	OW	34	18	2	13	-	32(11)	-	2	1	-	-	1	1(1)	1	-	-	-	1	1	-	9Y5b, 4Y1b, 1Y3b, 1Y10345, 1P1b	123
A04	30TXN996254	1025	A	OW	22	27	1	6	5	20	-	2	2	1	-	-	-	1	-	-	-	-	-	-	13Y5b, 3Y1b, 3Y3b, 4Y10345	110
A05	30TXN973264	1050	C-1	S	4	7	-	1	-	7	-	-	1	3	-	-	-	4	-	2	-	-	-	-	1Y5b, 2Y1b, 2Y10345, 2P5b	36
A06	30TXN010263	900	A	OW	7	11	-	2	-	3	-	-	1	-	1	1	-	4	-	-	-	-	-	-		30
A07	30TYN032290	1100	A	OW	45	37	2	10	4(1)	19(5)	-	13(6)	1	5	-	1	-	4	-	1(1)	4	2	-	1	5Y5b, 5Y1b, 3Y10345, 6P5b, 1P1b, 1P3b	170
A08	30TYN022291	950	A	OW	26	38	-	8	-	22(3)	-	7(1)	7	3	-	-	-	1	-	-	-	-	-	-	3Y5b, 4Y1b, 1Y3b, 2Y10345	122
A09	30TYN025309	1100	A	OW	9	30	1	7(1)	-	19(6)	1	5	1	4	-	-	-	6(2)	-	-	2	-	-	-	13Y5b, 8Y1b, 7Y10345, 1Y3b, 1P5b, 2P10345	117
A10	30TYN030308	1120	A	OW	13	20	-	1	-	6(3)	-	3	-	2	-	-	-	1	-	-	1	-	-	-	3Y5b,5Y1b,1P5b,4P1b	60
A11	30TYN031317	1000	A	O	2	17	-	8(2)	-	6	1	6(1)	2	-	-	-	-	2	-	2(1)	-	-	-	-	1Y1b,1Y10345,1Y03345	49
A12	30TYN029328	1050	A	O	1	14	-	3	-	10	-	5	1	-	-	-	-	2	-	1	-	-	-	-	2Y5b, 1P3b	40
A13	30TYN041355	1280	B	I	7	14	1	1	2(1)	45(11)	1	4(2)	2	6	-	1	1	7(1)	-	2	1	1	1	3	6Y5b, 1Y1b, 2P5b	109
A14	30TYN041376	1200	B	I	1	11	-	3	-	10(2)	-	-	5	1	-	-	1	3(1)	-	-	-	-	-	-	1Y5b	36
A15	30TYN040386	1300	C-1	S	4	4	-	1	1(1)	5(3)	-	-	6	3	-	-	-	6(1)	-	-	-	-	-	-		30
A16	30TYN045397	1250	B	O	13	4	-	-	-	1	1	-	6	3	-	-	-	1	-	2	-	-	-	-	4Y5b, 1P5b	36
A17	30TYN041392	1350	C-1	S	20	14	-	-	13	42(1)	1	1	2	3	-	1	6	39	2	5(2)	-	-	-	-	1Y5b, 1P5b, 1P3b	152
A18	30TYN032395	1450	D	I	43	18	-	1	12	20(2)	-	2(1)	15	6	-	-	6(1)	12(3)	-	-	1	2	1	7(1)	1Y5b,5P5b,2P10345	154
A19	30TYN033399	1500	D	O	117	29	3	-	4	1(1)	1	-	22	15	2	-	-	-	-	1	2	-	-	-		197

TABLE 10. Results of sampling *C. nemoralis* in the Esca Valley. Abbreviations as in Table 8.

Sample Nº.	U T M Coordinate	Altitude (m.)	Veget. zone	Biotope type	Yellow								Pink								Brown					
					00000		00300		12345		Others		00000		00300		12345		Others		00000		Banded			
					wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	wl	dl	Hyalozonate	Total
E01	30TXN614198	450	A	D	17	17	–	11(5)	–	14(5)	1(1)	1	1	10	3(2)	7(1)	1(1)	19(5)	1(1)	2	–	–	–	–	1P5b	106
E02	30TXN629211	475	A	O	5	8	–	–	1	4	–	–	–	4	–	–	–	5	1	2(1)	–	–	–	–	2Y5b	31
E03	30TXN632229	500	C-1	S	3	16	–	3(1)	4	17(6)	–	3	2	10	–	2	–	11	1	3	–	1	–	2(1)	1Y1b	79
E04	30TXN626247	525	C-2	O	3	9	–	–	–	6	–	3	–	4	–	–	–	3	–	–	–	–	–	–		28
E05	30TXN630264	550	A	O	14	33	–	3	8	13	12	11	4	3	1	1	3	1(1)	7(1)	8(1)	–	–	–	–	1P5b, 1P3b	124
E06	30TXN614268	650	A	I	–	21	–	3	–	10	–	3	2	3	–	1	–	3	–	4	–	–	–	–		50
E07	30TXN592265	750	A	O	37	121	5	6	3	12	2	13	4	10	3	1	1	1	1	6	–	–	–	–	1Y1b	227
E08	30TXN557270	900	A	O	22	26	–	–	1	3	–	–	–	1	–	–	–	–	–	–	–	–	–	–		53
E09	30TXN635269	550	A	D	1	8	–	1(1)	–	5	–	–	–	10	–	2	–	1	–	–	–	1	–	2		31
E10	30TXN628278	575	A	O	3	25	–	1	2	4	1	7(1)	–	2	–	6	–	3	–	9	–	2	1	1		67
E11	30TXN629301	600	C-1	S	–	–	3(1)	7(1)	1	8(2)	–	–	–	–	2(1)	2	1(1)	9(3)	–	–	–	–	–	1(1)		34
E12	30TXN633313	620	C-1	S	1	1	–	1	–	1	–	2	–	3	–	–	2(1)	4(1)	–	–	–	–	–	–		15
E13	30TXN635314	630	A	O	4	14	–	–	2	19	1(1)	1(1)	1	4	1	–	2	12(4)	–	4(1)	10	37	1	7(2)	3Y5b, 4P5b, 1P1b	131
E14	30TXN635337	640	A	OW	9	21	–	–	2	1	2	2	1	1	–	1	–	2	–	–	–	–	–	–	2Y5b, 2Y3b, 1P5b	47
E15	30TXN630376	680	A	OW	43	18	–	2	12	6	5(1)	7	8	4	–	–	1(1)	4	1	3	–	–	–	–	18Y5b,1Y1b,7Y3b,2P5b,2P3b	144
E16	30TXN626417	780	A	OW	3	14	–	3	1	8	–	3	–	3	–	–	–	3	–	–	–	–	–	–	1Y3b, 1P5b	40
E17	30TXN657348	670	A	O	23	88	2	–	1(1)	2(1)	3(1)	6(1)	–	10	–	1	–	1	2	6	–	3	–	–	1Y5b, 1Y1b, 2Y10345	152
E18	30TXN659356	660	A	OW	4	12	–	3	1(1)	5(1)	1	2	–	12	–	1	–	3(1)	–	–	–	–	–	–	2Y5b, 2Y3b, 1P5b, 1P3b	50
E19	30TXN658359	680	A	OW	6	13	1	1	4	8	2	1	1	2	–	–	–	1	–	–	–	–	–	–	3Y5b	43
E20	30TXN668369	720	A	O	49	11	–	3	1(1)	5	1(1)	–	–	3	–	–	–	–	–	–	–	1	1	–	1Y1b, 1P5b	77
E21	30TXN668391	700	C-2	OW	56	40	1	–	8	21	3	1	7	1	–	–	1	2	–	1	1	1	–	–	25Y5b,5Y3b,8P5b,2P10345	184
E22	30TXN692399	740	A	O	33	34	1	–	1	2	1	1	2	11	1	1	–	5	2	–	–	–	–	–	1P5b, 1P1b	97
E23	30TXN714391	780	A	OW	1	2	–	–	1	5(1)	–	–	1	1	–	1	–	2	1(1)	–	–	–	–	–	1Y5b, 1Y1b, 1P5b, 1P1b, 2P3b, 1P10345	22
E24	30TXN670409	700	A	OW	57	32	–	6	5	30(7)	3(2)	11(7)	10	6	–	–	–	4(2)	–	–	–	–	–	–	16Y5b, 3Y1b, 7Y3b, 1Y00340 1Y10345,4P5b,1P1b,3P3b, 2P10345	202

Sample Nº.	U T M Coordinate	Altitude (m.)	Veget. zone	Biotope type	Yellow 00000 wl	Yellow 00000 dl	Yellow 00300 wl	Yellow 00300 dl	Yellow 12345 wl	Yellow 12345 dl	Yellow Others wl	Yellow Others dl	Pink 00000 wl	Pink 00000 dl	Pink 00300 wl	Pink 00300 dl	Pink 12345 wl	Pink 12345 dl	Pink Others wl	Pink Others dl	Brown 00000 wl	Brown 00000 dl	Brown Banded wl	Brown Banded dl	Hyalozonate	Total
E25	30TXN676430	760	B	O	18	51	–	3	–	15(2)	–	1	1	17	1	2	7(1)	11(1)	4	5(1)	–	4	–	2	1Y5b,2Y10345,1P5b,1P1b	147
E26	30TXN678441	780	B	O	25	41	1	1	4	15	3(2)	5	6	7	–	1	1	7	–	5	–	–	–	–	4Y5b, 1Y1b, 1P5b	128
E27	30TXN682447	780	B	O	14	33	1	6	4(1)	12(1)	2(1)	2(1)	3	10	–	4(1)	–	6	–	3(1)	–	2	–	1	1P5b	104
E28	30TXN691461	760	B	D	2	11	–	3	–	9(4)	–	2	2	6	2	1(1)	1	7(5)	–	1	–	–	–	–	2P1b	49
E29	30TXN693472	800	C-1	S	–	5	–	1	–	11	–	–	–	2	–	1	–	5(1)	–	1	–	–	–	5(1)		31
E30	30TXN694489	800	C-1	S	–	–	–	3(1)	–	4	–	–	–	1	–	6(1)	–	6(1)	–	–	–	–	–	–		20
E31	30TXN674515	860	C-1	S	–	–	–	5	–	8(1)	–	1	–	–	–	5	–	5	–	2	–	–	–	–		26
E32	30TXN706486	820	C-1	S	4	14	–	1(1)	–	12(3)	–	2	1	7	–	5	–	35(4)	–	–	–	3	–	6(1)	1Y5b, 1P10345	92
E33	30TXN722498	920	C-3	I	1	6	1(1)	2(1)	–	5(1)	1(1)	–	–	2	–	2(1)	–	6(1)	–	–	–	–	–	–		26
E34	30TXN735496	1060	C-3	I	1	13	–	2	–	3(1)	–	3(3)	2	16	–	–	–	6	2(2)	2(2)	–	2	–	1	1Y1b	54
E35	30TXN758495	1100	C-3	I	5	9	–	6	–	5	–	4(4)	2	7	1	3	1(1)	5(2)	–	2(1)	–	–	–	–		50
E36	30TXN715499	880	C-3	I	1	13	–	–	1(1)	7	–	–	–	5	–	1	–	3	–	–	–	–	–	–		31
E37	30TXN723523	930	C-1	S	2	29	–	1(1)	–	12	–	–	–	12	–	6(1)	–	9	1	2(2)	–	–	–	–	1P5b	75
E38	30TXN757558	1070	C-3	I	2	11	–	–	1	3	–	–	1	1	–	–	–	1	–	–	–	–	–	–		20
E39	30TXN774557	1000	C-3	S	1	7	–	1(1)	–	1(1)	–	2(2)	4	9	2(1)	6(1)	1	14(1)	–	2(1)	1	5	1	1	1P1b	59
E40	30TXN748566	1200	D	I	2	8	–	–	–	4(2)	–	–	3	1	–	–	–	2(1)	–	–	–	–	1(1)	–		21
E41	30TXN815595	1700	D	S	1	7	–	–	1	14	–	–	–	9	–	–	–	12	–	–	–	3	–	1	1Y5b	49

TABLE 11. Results of sampling *C. nemoralis* in the Salazar Valley. Abbreviations as in Table 8.

Sample Nº.	U T M Coordinate	Altitude (m.)	Veget. zone	Biotope type	Yellow 00000 wl	Yellow 00000 dl	Yellow 00300 wl	Yellow 00300 dl	Yellow 12345 wl	Yellow 12345 dl	Yellow Others wl	Yellow Others dl	Pink 00000 wl	Pink 00000 dl	Pink 00300 wl	Pink 00300 dl	Pink 12345 wl	Pink 12345 dl	Pink Others wl	Pink Others dl	Brown 00000 wl	Brown 00000 dl	Brown Banded wl	Brown Banded dl	Hyalozonate	Total
S01	30TXN490284	600	A	O	35	15	–	–	1	1	3	3	3	22	–	–	3	2	–	2	–	–	–	–	1Y5b, 1P3b	92
S02	30TXN473295	750	A	O	3	29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		32
S03	30TXN487299	700	A	O	17	52	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		89
S04	30TXN497291	500	A	O	8	21	–	7	–	1	–	7	1	9	1	9	–	2	–	2	–	–	–	–		66
S05	30TXN499306	630	A	O	25	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		30
S06	30TXN548329	480	A	D	–	13	1	–	3	3	–	–	–	6	–	–	1	5	–	1	–	1	–	–		34
S07	30TXN554368	620	A	O	20	31	1	1	–	1	1	1	1	1	–	–	1	–	–	2	–	–	–	–		61
S08	30TXN558383	640	B	O	15	15	–	–	2	4	1	4	4	2	–	1	1	–	–	3	–	–	–	–	1Y3b	53
S09	30TXN557426	660	B	O	14	16	–	–	1	1	–	3	1	4	–	–	–	2	–	–	–	–	–	–	2Y3b, 2Y10345,1P10345	47
S10	30TXN551457	670	B	I	–	23	–	1	–	4	–	4	1	9	–	–	–	4	–	3	2	1	–	–		52
S11	30TXN562496	780	C-2	I	21	15	–	1	3	1	1	1	3	6	1	–	3	–	1	2	–	–	–	–		55

ers) and is better adapted than pink to inversions of temperature (Cain *et al.,* 1969).

Correspondingly, the montane zone of Canfranc and Esca is characterized by an increase in pink shell frequency. This pattern is not observed in Caldarés which shows a high frequency of yellow. Such differences might be related to the lack of oceanic influence in Caldarés. This last explanation is probably not the only one as no differences have been found between the montane zone of Esca and Canfranc, in spite of a more marked oceanic influence in Esca.

The situation with respect to the banding system is more complex. No general trend for banding has been found. In the Canfranc Valley the frequency of banded shells increases with altitude, in Caldarés the frequency of five-banded shells is high only in the sub-alpine zone, in Esca there are no clear variations which might be related to altitude or vegetational zonation and in the Salazar Valley unbanded shells scarcely vary in frequency except for those samples from hillsides in the lower part of the valley (Figs. 3, 5, and 7). These results are quite different from those found in the Central Pyrenees, where as a whole unbanded shells are more frequent at lower and higher altitudes. This led Lamotte (1951) and Arnold (1968, 1969) to suggest that unbanded snails are at an advantage in climatically extreme conditions. The heterogeneity of the results in our area and the fact that unbanded shells predominate in the intermediate part of the Ter Valley (Eastern Pyrenees; Jones & Irving, 1975) do not seem to confirm this hypothesis, as the frequency of unbanded shells is not apparently associated either with climatic zones or with vegetation zones.

Thus, while climatic factors may influence the distribution of the polymorphism for shell colour in our valleys, they are not apparently related to banding, supporting the statement of Jones & Irving (1975) that the polymorphism of *C. nemoralis* does not fall within the scope of simple climatic interpretations.

B. Variation of morph frequencies with biotope

As revealed by the PCA the major feature of the variation of the polymorphism of *C. nemoralis* in the Western Pyrenees is the relationship between biotope and phenotypic composition of the colonies. All the colonies with the highest frequencies of pink and banded shells correspond to the most shaded and covered biotopes and on the contrary the most open, dry and sunny localities with scarce vegetation of small box bushes and furze have the highest frequencies of yellow and unbanded shells. The first kind of biotope has average frequencies of yellow and unbanded of 36% and 20% respectively and the second kind of 84% and 78% respectively. Intermediate biotopes have intermediate overall frequencies of these characters. Consistent with this pattern of variation are the results found in the sub-alpine zone of Canfranc, Esca and Caldarés, which correspond to biotopes other than the two ones above mentioned. Thus, yellow and unbanded are at higher frequencies in Canfranc (open biotope) than in Esca and Caldarés (sub-alpine biotopes more shaded).

Two main hypotheses have been proposed to account for the relationship between morph frequency and biotope, one relative to selective predation and the other relative to microclimatic factors.

There is no evidence that selective predation by thrushes is an important agent in our area as very few shells broken were found and, in addition, whereas the distribution of colour (brown and pink are more frequent in shaded biotopes) is congruent with the hypothesis of visual selection for crypsis (Cain & Sheppard, 1950, 1954; Currey *et al.,* 1964), the distribution of banding is different from that expected under this hypothesis, as unbanded morphs are not more frequent in shaded biotopes but in open ones.

The kind of variation with biotope here described is more compatible with the explanation of Lamotte (1959, 1966) about the differences in the thermal properties of the shells, that is, darker shells are expected to be favoured in shaded biotopes. The experimental results on artificial resistance of the morphs performed by Boettger (1954) and Lamotte (1966), though not very conclusive, agree quite well with this explanation and the field distribution of the morphs in the studied valleys.

Similar results (with yellow and unbanded frequent in insolated and warm places and pink and banded snails frequent in sheltered environments) have been found in the Garona and Segre-Valira Valleys (Arnold, 1968).

Clarke (1960) found in the Oxford district that the closely related species *C. hortensis* responds to habitat in the same way as described here. Although he interpreted these

results in terms of selective predation, this author suggests (Clarke *et al.,* 1978) that microclimatic factors cannot be excluded. Bengtson *et al.* (1976) describe the same relation from Iceland. They suggest that unbanded is favoured in places where radiation temperature fluctuates over a wide amplitude since they reflect a larger portion of the heat energy than do banded individuals. The experimental results of Steigen (1979) support this hypothesis. He shows that yellow unbanded and yellow banded morphs have significantly different strategies of metabolic response to temperature. Unbanded are more able to regulate metabolism down to an economic level at low temperature but also are more capable of deriving the profit of an increase in temperaure from insolation because they need only half of the energy subsidies to get the same gain in metabolic rate as banded. Thus, unbanded yellow seems to be better adapted in exposed sites with higher temperature amplitudes whereas yellow banded, especially those with fused banding, are better adapted to areas where temperature amplitudes are relatively small.

Although the experiments of Steigen were carried out on *C. hortensis* a similar response ought to be expected in *C. nemoralis* as the two species respond in a similar way to the nature of the biotopes, with darker shells in shaded places and lighter shells in open ones (Clarke, 1962). This seems to be confirmed by the results on differential mortality of Richardson (1974) and Tilling (1983).

Ranges of daily oscillations in temperature and other environmental factors within the biotope are greatly influenced by the characteristics of the vegetation (shape, density, etc.) Our samples relate to these characteristics in three ways: 1) The frequency of yellow unbanded snails increases with decreasing shade and density of vegetation; 2) Shaded and dense biotopes are the only ones with high frequencies of fused bands, and the frequency of fusions increases with increasing density of vegetation; 3) The higher frequencies of hyalozonate bands (recessive allele that suppresses the colour of the bands in such a way that the shell appears phenotypically nearly as unbanded) are found in exposed biotopes where unbanded condition seems to be at an advantage. Because of the simultaneous occurrence in open biotopes of the widest part of the Canfranc and Esca Valleys of a decrease of unbanded and an increase of hyalozonate (Table 7), it might be thought that these two facts are related in some way, that is, hyalozonate might act as a counterbalance for the relative decrease of unbanded (53%) if hyalozonate banded (18%) is considered as effectively unbanded with respect to the biotope. In this way, it must be pointed out that if hyalozonate is included with unbanded, then "effective" unbanded (71%) in these samples would approach closely the frequencies of unbanded (78%) in the samples of common open biotopes. Of the other phenotypes that might be considered as effectively unbanded (00300, 00345) only mid-banded has higher frequencies in the wider part of the Canfranc Valley than in other parts of it, but this pattern is not found in Esca.

In the samples above mentioned hyalozonate is almost equally distributed between yellow and pink, and the excess of unbanded among yellow disappears (Table 7).

Similar results in the relationship between yellow and open biotopes and fusions and shaded ones have been recorded by Lamotte (1959) for several regions of France, by Arnold (1968, 1969, 1970) in the Eastern Pyrenees and in Touraine, by Richards & Murray (1975) in an introduced colony of *C. nemoralis* at Virginia, by Sacchi (1981) in Galicia, and by Bengtson *et al.* (1976) in *C. hortensis.* These relationships tend to reduce the polymorphism for shell colour and banding within the colonies. Other factors such as the mobility of the snails (although reduced) may be sufficient to promote a genetic flow among neighbouring colonies that restores part of the variability lost by selection.

Other characters than shell colour and banding, i.e. white lip, punctate bands and other variants of the number of bands, do not seem to have any relation with climatic factors or biotope. In particular white lip is not found preferentially in wetter environments as Jones *et al.* (1977) have pointed out, a question that will be treated in a future paper. What is seen is that these morphs show an irregular pattern of variation with great differences in frequency between neighbouring colonies, a fact that may be taken as an indication of selectively neutral values for these rare alleles, as their frequencies seem to be the result of stochastic processes. An alternative point of view might include the existence of other (cryptic ?) factors than those considered here directly favouring these alleles in a few colonies.

These unexplained variations support the

suggestion of Jones *et al.* (1977) that the polymorphism of *Cepaea* cannot be explained by simplistic models based on only one or two kinds of selection. Morph frequencies appear to be the result of several evolutionary forces whose equilibrium varies from population to population, thus giving a heterogeneous pattern of this polymorphism.

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REFERENCES CITED

ARNOLD, R. W., 1968, Climatic selection in *Cepaea nemoralis* (L.) in the Pyrenees. *Philosophical Transactions of the Royal Society of London,* ser. B, 253: 549–593.

ARNOLD, R. W., 1969, The effects of selection by climate on the land snail *Cepaea nemoralis* (L.). *Evolution,* 23: 370–378.

ARNOLD, R. W., 1970, A comparison of populations of the polymorphic land snail *Cepaea nemoralis* (L.) living in a lowland district in France with those in a similar district in England. *Genetics,* 64: 589–604.

ARNOLD, R. W., 1971, *Cepaea nemoralis* on the East Sussex South Downs and the nature of area effects. *Heredity,* 26: 277–298.

BENGTSON, S.-A., NILSSON, A., NORDSTOM, S. & RUNDGREN, S., 1976, Polymorphism in relation to habitat in the snail *Cepaea hortensis* in Iceland. *Journal of Zoology,* 178: 173–188.

BOETTGER, C. R., 1954, Zur Frage der Verteilung bestimmter Varianten bei der Landschneckengattung *Cepaea* Held. *Biologisches Zentralblatt,* 73: 312–333.

CAIN, A. J., CAMERON, R. A. D. & PARKIN, D. T., 1969, Ecology and variation of some helicid snails in Northern Scotland. *Proceedings of the Malacological Society of London,* 38: 269–299.

CAIN, A. J., KING, J. M. B. & SHEPPARD, P. M., 1960, New data on the genetics of polymorphism in the snail *Cepaea nemoralis* (L.). *Genetics,* 45: 393–411.

CAIN, A. J. & SHEPPARD, P. M., 1950, Selection in the polymorphic land snail *Cepaea nemoralis. Heredity,* 4: 275–294.

CAIN, A. J. & SHEPPARD, P. M., 1954, Natural selection in *Cepaea. Genetics,* 39: 89–116.

CAIN, A. J. & SHEPPARD, P. M., 1957, Some breeding experiments with *Cepaea nemoralis* (L.). *Journal of Genetics,* 55: 195–199.

CAMERON, R. A. D., 1969, The distribution and variation of three species of land snail near Rickmansworth, Hertfordshire. *Zoological Journal of the Linnean Society,* 48: 83–111.

CAMERON, R. A. D., CARTER, M. A. & HAYNES, F. N., 1973, The variation of *Cepaea nemoralis* in three Pyrenean valleys. *Heredity,* 31: 43–74.

CARTER, M. A., 1968, Area effects and visual selection in *Cepaea nemoralis* (L.) and *Cepaea hortensis* (Müll.). *Philosophical Transactions of the Royal Society of London,* ser. B, 253: 397–446.

CLARKE, B., 1960, Divergent effects of natural selection on two closely-related polymorphic snails. *Heredity,* 14: 423–443.

CLARKE, B., 1962, Natural selection in mixed populations of two polymorphic snails. *Heredity,* 17: 319–345.

CLARKE, B., ARTHUR, W., HORSLEY, D. T. & PARKIN, D. T., 1978, Genetic variation and natural selection in pulmonate molluscs. In FRETTER, V. & PEAKE, J., eds., *Pulmonates,* vol. 2A, Systematics, Evolution and Ecology. Academic Press, London, New York, San Francisco, p. 219–270.

CURREY, J. D., ARNOLD, R. W. & CARTER, M. A., 1964, Further examples of variation of populations of *Cepaea nemoralis* with habitat. *Evolution,* 18: 111–117.

GUERRUCCI-HENRION, M. A., 1966, Recherches sur les populations naturelles de *Cepaea nemoralis* en Bretagne. *Archives de Zoologie Expérimentale et Générale,* 107: 369–417.

HARVEY, P. H., 1971, Populations of *Cepaea nemoralis* from Southwestern France and Northern Spain. *Heredity,* 27: 353–368.

JONES, J. S. & IRVING, A. J., 1975, Gene frequencies, genetic background and environment in Pyrenean populations of *Cepaea nemoralis* (L.). *Biological Journal of the Linnean Society,* 7: 249–259.

JONES, J. S., LEITH, B. H. & RAWLINGS, P., 1977, Polymorphism in *Cepaea:* A problem with too many solutions? *Annual Review of Ecology and Systematics,* 8: 109–143.

LAMOTTE, M., 1951, Recherches sur la structure génétique des populations naturelles de *Cepaea nemoralis* (L.). *Bulletin Biologique de la France et de la Belgique* (Suppl.), 35: 1–239.

LAMOTTE, M., 1954, Sur le déterminisme génétique du polymorphism chez *Cepaea nemoralis* L. *Compte Rendus de l'Academie des Sciences de Paris,* 239: 365–367.

LAMOTTE, M., 1959, Polymorphism of natural populations of *Cepaea nemoralis. Cold Spring Harbor Symposia on Quantitative Biology,* 24: 65–86.

LAMOTTE, M., 1966, Les facteurs de la diversité du polymorphisme dans les populations naturelles de *Cepaea nemoralis* (L.) (Gastropoda, Pulmonata). *Lavori della Società Malacologica Italiana,* 3: 33–73.

LAMOTTE, M., 1968a, Les traits généraux du polymorphisme de la coquille dans les populations naturelles de *Cepaea nemoralis* (Mollusques Helicidae) des Pyrénées françaises. *Compte Rendus de l'Academie des Sciences de Paris,* 267d: 1318–1321.

LAMOTTE, M., 1968b, Influence de l'altitude sur la frequence du charactère "absence de bandes" dans les populations de *Cepaea nemoralis* (Mollusques Helicidae) des Pyrénées françaises. *Compte Rendus de l'Academie des Sciences de Paris,* 267d: 1649–1652.

LEBART, L. & FÉNELON, J. P., 1975, *Statistique et informatique appliquées.* Bordas Ed. Paris, Bruxelles, Montreal.

MONTSERRAT, P., 1971, El ambiente vegetal jacetano. *Pirineos,* 101: 5–22.

RICHARDS, A. V. & MURRAY, J., 1975, The relation of phenotype to habitat in an introduced colony of *Cepaea nemoralis. Heredity,* 34: 128–131.

RICHARDSON, A. M. M., 1974, Differential climatic selection in natural populations of land snail *Cepaea nemoralis. Nature,* 247: 572–574.

SACCHI, C. F., 1981, Convergence du polymorphisme chez *Cepaea nemoralis* (L.) et *Monachoides inchoatus* (Mor.) (Gastropoda Pulmonata): parallélisme écologique ou mimétisme imitatif?. *Atti della Società italiana di Scienze naturale Museo civico di Storia naturale di Milano,* 122: 139–150.

SOLER-SAMPERE, M. & PUIGDEFÁBREGAS, C., 1972, Esquema litológico del Alto Aragón occidental. *Pirineos,* 106: 5–15.

STEIGEN, A. L., 1979, Temperature effects on energy metabolism in banded and unbanded morphs of the snail *Cepaea hortensis* Müll. *Oecologia* (Berl.), 141: 163–173.

TILLING, S. M., 1983, An experimental investigation of the behavior and mortality of artificial and natural morphs of *Cepaea nemoralis* (L.). *Biological Journal of the Linnean Society,* 19: 35–50.

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MOVEMENT AND GENE FLOW IN *PARTULA TAENIATA*

James Murray[1] & Bryan Clarke[2]

ABSTRACT

The land snail *Partula taeniata* is the most abundant and widespread member of its genus inhabiting the island of Moorea in French Polynesia. Local populations are differentiated in the size and shape of the shell and the frequencies of genes controlling color and banding. In order to determine the amount of gene exchange between populations, two experiments were performed. In the first, a 10 × 10 m square was divided into 25 quadrats, and the snails were marked to show their initial locations. Movements were recorded over an initial period of nine weeks, with a subsequent collection after five years. The mean radius of individual movements rose to 219 cm over the initial period, with the standard deviation increasing faster than the mean. The maximum displacement was 737 cm. Recaptures after five years established the longevity of *P. taeniata* in nature and showed that individuals may remain for long periods close to their original points of capture.

In the second experiment, genetically marked individuals were introduced into a natural population. Samples taken after one year and thirteen years show that the introduction was successful and that the genes spread from the point of introduction. After thirteen years no marked animals remained in the population, but the mean displacement of the introduced genes had risen to 1059 cm with a maximum recorded distance of 27 m. These data indicate that gene flow in *P. taeniata* is sufficient to prevent the local decay of variability but cannot be expected to prevent local differentiation in response to environmental pressures.

INTRODUCTION

The role of gene flow in the genetic constitution of species remains an open question. The classical view, most clearly articulated by Mayr (1963), is that gene flow is responsible both for the integration of the species' gene pool and for the prevention of local adaptation at the edge of the species range. This model of genetic structure has the attractive feature of explaining why species do not continue to expand their ranges by further adaptation of their peripheral populations.

The classical view has been challenged from two different directions. First, it has been suggested that adaptation and differentiation may often proceed in the face of extensive gene flow. Both theoretical studies (Clarke, 1966; Endler, 1977) and experimental ones (Bradshaw, 1960; McNeilly & Antonovics, 1968; Pimentel, Smith & Soans, 1967) show that significant differentiation can take place in the absence of complete isolation. The second challenge has come from those who maintain that gene flow is in fact not very great, certainly not of the magnitude required to preserve the homogeneity of species on a continental scale (Ehrlich & Raven, 1969; Levin & Kerster, 1974; Harper, 1977).

Although the idea of severely restricted gene flow has been widely accepted (e.g. Grant, 1980), the current literature suggests that a reaction has begun. Some classical estimates of dispersal have recently been drastically revised. For example, in their study of *Drosophila pseudoobscura,* Dobzhansky & Wright (1943) recorded a maximum displacement of 500 m. While they recognized that individual flies might disperse well beyond this distance, they could hardly have anticipated the results of Jones *et al.* (1981) and Coyne *et al.* (1982), who showed that *D. pseudoobscura* can move 15 km in 15 hours, leaving favorable habitats and crossing desert terrain to do so.

Another case in which gene flow has been underestimated is that of *Mus musculus* (Baker, 1981). The conventional model of house mouse populations assumes very small, isolated demes in which inbreeding groups defend their home ranges, driving away or killing potential immigrants (Lewontin & Dunn, 1960; Petras, 1967). Baker, however, has shown with genetic markers that

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alleles spread quickly and that within one or two generations they can reach populations more than 60 m away. Most of the dispersal is accomplished by the offspring of introduced individuals, rather than by the individuals themselves.

These studies suggest several aspects of dispersal that have generally been neglected. First, it is important to distinguish between gene flow and the movement of individuals. Immigrants may not affect the gene pool of recipient populations unless they are successful in reproducing. On the other hand, the studies of house mice indicate that genes may be transmitted effectively despite low individual mobility between populations.

A second point is that there are two rather different ways in which gene flow may influence the genetic structure of populations. Conventional models represent the change of gene frequency as a function of differences in gene frequencies between donor and recipient populations, and of the proportion of immigrants in the recipient population. However, one can also think of gene flow as a process of injecting new variation into a population, a process analogous to mutation. From this point of view the form of the distribution of dispersal distances becomes important. The usual finding is that dispersal distances are highly leptokurtic (Endler, 1977; Harper, 1977). Extreme values, which are those most likely to be missed, may therefore be of great biological significance.

These considerations have led us to reexamine information that we have obtained from marking experiments in populations of *Partula taeniata* Mörch over the past 20 years.

METHODS

The Animal: Partula taeniata is one of several species of this genus of land snails inhabiting the island of Moorea in French Polynesia. As well as varying in the color and banding of the shell (Murray & Clarke, 1976) and in allozymes (Johnson, Clarke & Murray, 1977), *P. taeniata* varies geographically in its size and proportions (Crampton, 1932; Murray & Clarke, 1968a). With the exception of the narrow coastal zone, *P. taeniata* is distributed in forest habitats over the whole island (Crampton, 1932; Murray & Clarke, 1980) and shows an association with small shrubs (Murray, Johnson & Clarke, 1982). In dry weather the snails remain attached to the undersides of leaves, while at the first hint of rain they emerge and move about actively. Rainfall is high throughout the year, although the period from December to February (summer) has the greatest precipitation.

Marking: Partula can be marked by drilling small holes just behind the lip of the shell. The animal repairs such holes by depositing shell from the inside, leaving a tiny pit, often of a contrasting color.

The Experiments: Our information on dispersal and gene flow in *Partula taeniata* comes from two experiments carried out over an extended period from 1962 to 1980.

The first experiment, begun in 1962, was the more detailed, but it measures dispersal only. In the central portion of Faatoai Valley in northwest Moorea a grid of twenty-five 2 × 2 m squares was established. The area was an open grove of *Adenanthera* saplings 3–6 m high beneath a canopy of larger trees. Animals were marked according to their locations on the grid. Every snail in the sixteen squares of the outermost ring was given an identical mark. Those in each of the eight squares of the middle ring were given a mark indicating the individual square. In the central square unique marks were assigned to every individual sapling in the square (Fig. 1).

The grid was searched at weekly intervals for nine weeks. All unmarked animals were marked on each occasion, and the locations of all animals were recorded. On the final sampling date, the eight 10-m squares adjoining the study area were searched, and the locations of all marked animals were recorded. In 1967, further collections were made from the same area.

The second experiment was designed to monitor the spread of genetic markers in natural populations. It was begun in 1967 in three different locations, two in Faatoai Valley and one in Opunohu Valley. These populations had been sampled previously to make sure that they did not contain the N4 phenotype, a dark brownish purple shell color, dominant in expression, produced by an allele at the color locus (Murray & Clarke, 1976). The allele (C^{N4}) was then introduced into the populations.

Two of the three introductions were unsuccessful. In the third case, in Faatoai, 30 pale animals were removed from the population. Eight of these came from a coconut palm in the center of the area, seven within 5 m of the tree, and the remaining fifteen within 10 m. They were replaced with 30 N4 animals from upper Faatoai, about 700 m from the site of introduction. All the animals were released

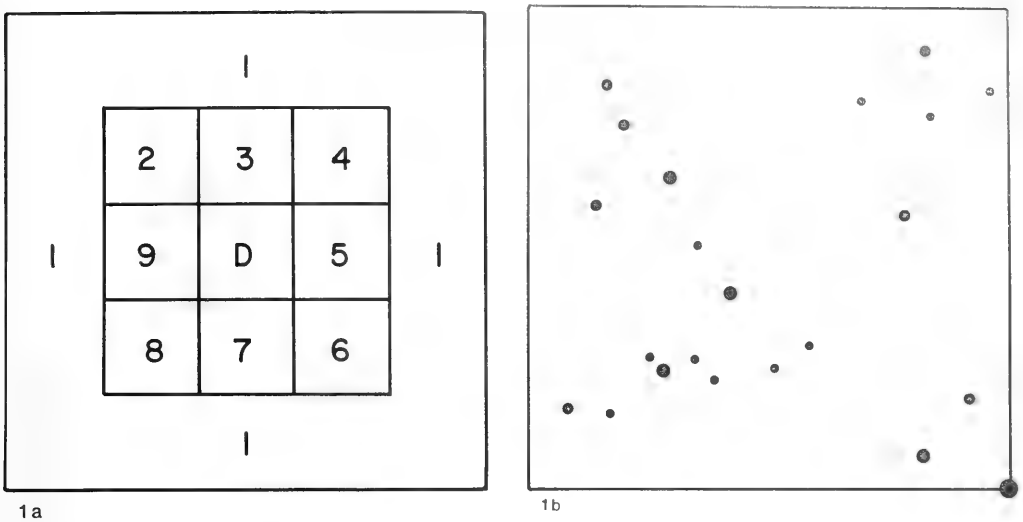

FIG. 1. Design of the first experiment. a. 10 × 10 m square divided into 25 2 × 2 m quadrats. Animals from the 16 outer quadrats were given a single identical mark (1). Those from the middle ring were numbered according to the individual quadrat (2–9). Those from the center quadrat (D) were marked to indicate the plant on which they were found. b. The distribution of *Adenanthera* saplings in the central 2 × 2 m quadrat (D).

on the central palm tree. The population was sampled in 1968 and again in 1980. On each occasion a distance of 10 m was searched beyond the furthest N4 individual detected.

RESULTS

In the first experiment 1025 individuals were marked over the course of the experiment. The proportion of marked animals rose rapidly to reach a steady recapture rate of about 88%.

The most detailed information comes from the animals marked on individual saplings of the central area. Fig. 2 shows the results for two representative saplings. On the upper right is sapling #12. Three animals were marked here on the first census with a total of seven over the whole period. At the second census all three of the original animals had moved, two to one adjacent sapling and one to another. One stayed at the new location; two returned to sapling #12. The only other recorded move came in week five, when an animal moved to a third adjacent sapling.

Snails from sapling #15 in the left center show a more complicated history of gradual spreading over time. Seven individuals were marked initially, and no other unmarked animals appeared on that sapling thereafter. In the first week, one of the closest saplings was reached. In the second, another step was taken. Three other saplings were reached in weeks four and five. Then in week six, one animal moved into the adjacent 2-m square. Another animal moved to one additional sapling in week eight. Finally, in 1967 one of the original animals was found in the outer ring, two squares from its original position.

Fig. 3 shows a composite diagram of all the movement recorded in 1962 for animals originally marked in the central square. The lines indicate the *minimum* movements necessary to account for all the recaptures.

There are several ways in which to treat these data. First, we can consider each sapling as the center of an expanding circle and calculate the growth, with time, of the largest displacement from each sapling, averaged over saplings. This statistic rises from 43 cm after one week to 219 cm after eight weeks. The increase is, however, rather erratic, with the standard deviation rising faster than the mean (Table 1). There is a burst of activity between weeks four and five when the mean radius more than doubles and the standard deviation is quadrupled. The increase was not accompanied by any obviously unusual weather patterns.

A second way to read the data is to consider the recaptures in the final sample from outside the marking area. A sample of 1496

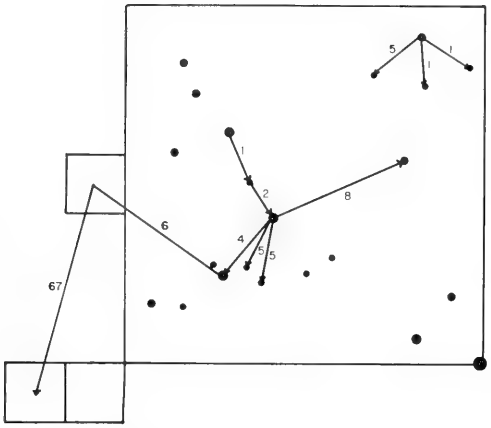

FIG. 2. Movement of animals first marked on sapling #12 (upper right) and sapling #15 (left center). Only recaptures that expanded the range of observed movements are recorded. The numbers indicate the weeks in which new movements took place. "67" indicates a recapture in 1967. Small squares represent the surrounding 2 × 2 m squares, at a reduced scale.

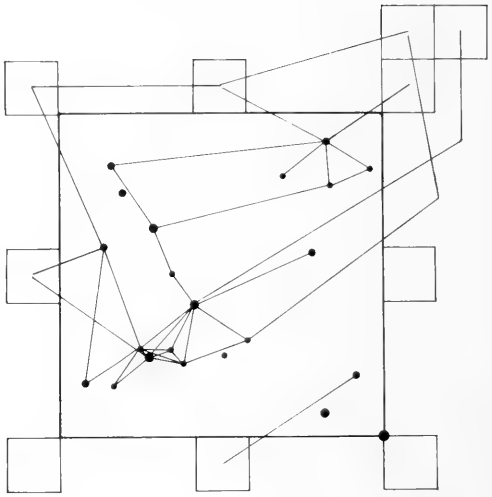

FIG. 3. A composite diagram of all movements recorded in 1962 for animals marked in the central quadrat. Lines indicate the *minimum* movements necessary to account for all recaptures. Small squares represent the surrounding 2 × 2 m squares, at a reduced scale.

TABLE 1. Increase in the mean radius of dispersal with time during the first experiment. $\bar{x}$ is calculated from the largest observed displacement from each sapling, averaged over saplings.

Week	$\bar{x}$	S_x
1	43.1 cm	20.2 cm
2	56.0	33.0
3	80.6	54.0
4	87.1	50.6
5	196.5	218.4
6	202.6	216.0
7	202.6	216.0
8	219.1	230.8

animals was found in the eight surrounding 10-m squares. Of these 38 (2.5%) were recaptures. In every case the marks were from the outermost ring of quadrats. The mean distance of marked animals from the boundary was 42 cm with a maximum of 2 m.

Finally, and most importantly for gene flow, there is simply the maximum recorded displacement in the experiment. This is the greatest distance of any recapture from the site where the animal was marked. One of the animals from the central square moved, during the nine week period, a distance of 737 cm from its initial position.

Fig. 4 shows the recaptures in 1967 of

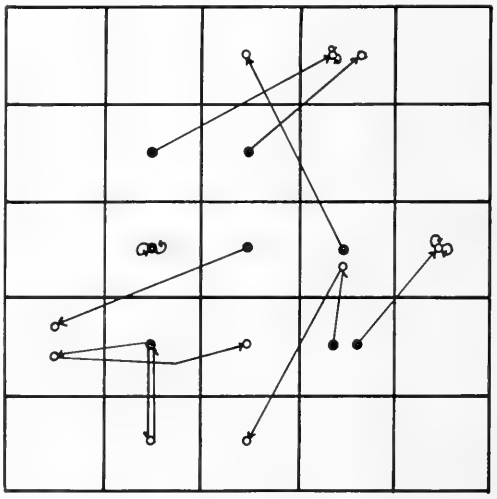

FIG. 4. Movements of eight animals marked in 1962 in the central quadrat and recaptured in 1967. Filled circles indicate sites of initial capture. Open circles show sites of recapture in 1967. Five additional animals, first marked in the outer ring, were recaptured but cannot be assigned to an initial quadrat.

animals originally marked in the center and in the middle ring. In addition to these eight, five animals from the outer ring were also found, but these cannot be assigned to an individual initial square.

The second experiment, in which the C^{N4} allele for brownish purple color was introduced, in 1967, into an entirely pale population, was sampled in 1968 and 1980. The results are shown in Figs. 5 and 6. In 1968, 126 animals were captured, of which 21 carried the introduced allele. Ten of these were marked recaptures, while eleven were umarked and must have been offspring of the original immigrants.

The mean distance from the point of introduction was almost identical for recaptures and their offspring: 342 cm and 352 cm respectively, with an overall mean of 347 cm and a standard deviation of 331 cm. The maximum recorded movement is 10 m.

Fig. 6 shows the distribution of genetically marked animals in 1980. No physically marked animals remained, but the genes are still present and continue to spread. Thus we are able to see the chaining effect of gene flow over the generations. The distribution is asymmetrical, with movement mostly in the direction of the more favorable habitats (as indicated by the density of resident animals). The distances of genetically marked animals from the point of introduction have increased to a mean of 10.6 m and a standard deviation of 6.3 m. The maximum recorded distance has risen to 27 m. Interestingly enough, neither the 1968 nor the 1980 sample shows the kurtosis so often found in data on dispersal. The coefficients of kurtosis are 2.5 and 3.3 respectively (where the kurtosis of a normal distribution = 3).

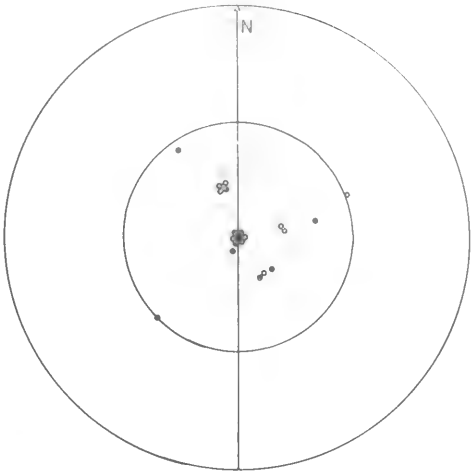

FIG. 5. Second experiment: Distribution of N4 animals in 1968. Open circles indicate recaptures. Solid circles are unmarked animals, offspring of those that were introduced. The two rings have radii of 10 and 20 m respectively.

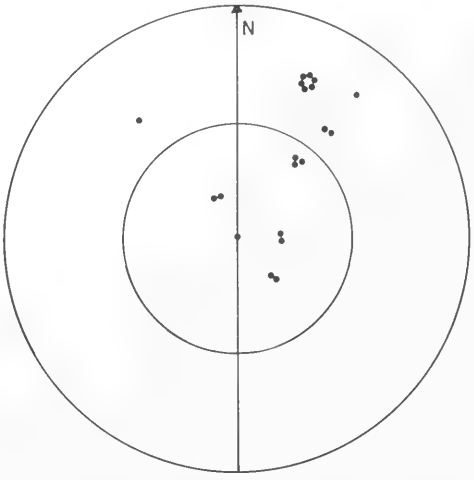

FIG. 6. Second experiment: Distribution of N4 animals in 1980. No marked animals remain. The two rings have radii of 10 and 20 m respectively.

DISCUSSION

These data clearly indicate that movement in *P. taeniata* is very restricted, even when observations are extended over long periods of time. Although the animals do not seem to "home", they only disperse very slowly from their birthplaces. The mean radius of dispersal appears to increase, in the long term, proportionally to the square root of time. Hence even on a small island such as Moorea, the populations of *P. taeniata* are likely to be integrated by gene flow only on a scale of tens of thousands of years. It is therefore not surprising to find differentiation among populations on the island.

Nevertheless, it is also apparent that gene flow can have important effects at the local level. In the absence of significant kurtosis we can apply Wright's estimate of the neighborhood size for these animals: $\pi(2\sigma)^2$, where σ is the standard deviation of dispersal per generation (Wright, 1946). The natural generation time of *P. taeniata* must lie between our two samples from the second experiment at one and thirteen years. The evidence here and from other experiments indicates that *P. taeniata* can reach maturity within a year and that adult mortality is approximatey 50% per annum. These figures

suggest an average generation time of two to three years. Therefore σ can be estimated to be between 3.31 and 6.33 m, say 4 m conservatively, indicating a neighborhood size of approximately 200 m^2. Since the density of *P. taeniata* in good habitats is of the order of two to ten individuals per m^2, then effective population sizes are approximately 400 to 2000. Thus the populations are often large enough to be principally affected by systematic evolutionary forces, if the forces are such that selective values are greater than about 10^{-3}.

These results are consistent with our studies of speciation in *Partula* (Clarke, 1968; Murray & Clarke, 1968b, 1980; Clarke & Murray, 1969; Johnson, Clarke & Murray,1977). The local differentiation of populations and the development of circular overlaps indicate that gene flow permits an adaptive response to local environmental pressures, even over distances as little as 10 m, without substantial loss of genetic variation.

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LITERATURE CITED

BAKER, A. E. M., 1981, Gene flow in house mice: introduction of a new allele into free-living populations. *Evolution,* 35: 243–258.

BRADSHAW, A. D., 1960, Population differentiation in *Agrostis tenuis* Sibth. III. Populations in varied environments. *New Phytologist,* 59: 92–103.

CLARKE, B., 1966, The evolution of morph-ratio clines. *American Naturalist,* 100: 389–402.

CLARKE, B., 1968, Balanced polymorphism and regional differentiation in land snails. In DRAKE, E. T., ed., *Evolution and Environment.* Yale University, New Haven, Connecticut, p. 351–368.

CLARKE, B. & MURRAY, J., 1969, Ecological genetics and speciation in land snails of the genus *Partula. Biological Journal of the Linnaean Society of London,* 1: 31–42.

COYNE, J. A., BOUSSY, I. A., PROUT, T., BRYANT, S. H., JONES, J. S. & MOORE, J. A., 1982, Long-distance migration of *Drosophila. American Naturalist,* 119: 589–595.

CRAMPTON, H. E., 1932, Studies on the variation, distribution, and evolution of the genus *Partula.* The species inhabiting Moorea. *Carnegie Institution of Washington Publications,* 310: 1–335.

DOBZHANSKY, T. & WRIGHT, S., 1943, Genetics of natural populations. X. Dispersion rates in *Drosophila pseudoobscura. Genetics,* 28: 304–340.

EHRLICH, P. R. & RAVEN, P. H., 1969, Differentiation of populations. *Science,* 165: 1228–1232.

ENDLER, J. A., 1977, *Geographic variation, speciation, and clines.* Princeton University, Princeton, New Jersey, ix + 246 p.

GRANT, V., 1980, Gene flow and the homogeneity of species populations. *Biologisches Zentralblatt,* 99: 157–169.

HARPER, J. L., 1977, *Population biology of plants.* Academic Press, New York, xxiv + 892 p.

JOHNSON, M. S., CLARKE, B. & MURRAY, J., 1977, Genetic variation and reproductive isolation in *Partula. Evolution,* 31: 116–126.

JONES, J. S., BRYANT, S. H., LEWONTIN, R. C., MOORE, J. A. & PROUT, T., 1981, Gene flow and the geographical distribution of a molecular polymorphism in *Drosophila pseudoobscura. Genetics,* 98: 157–178.

LEVIN, D. A. & KERSTER, H. W., 1974, Gene flow in seed plants. *Evolutionary Biology,* 7: 139–220.

LEWONTIN, R. C. & DUNN, L. C., 1960, The evolutionary dynamics of a polymorphism in the house mouse. *Genetics,* 45: 705–722.

MAYR, E., 1963, *Animal species and evolution.* Harvard University, Cambridge, Massachusetts.

McNEILLY, T. & ANTONOVICS, J., 1968, Evolution in closely adjacent populations. IV. Barriers to gene flow. *Heredity,* 23: 205–218.

MURRAY, J. & CLARKE, B., 1968a, Inheritance of shell size in *Partula. Heredity,* 23: 189–198.

MURRAY, J. & CLARKE, B., 1968b, Partial reproductive isolation in the genus *Partula* (Gastropoda) on Moorea. *Evolution,* 22: 684–698.

MURRAY, J. & CLARKE, B., 1976, Supergenes in polymorphic land snails. I. *Partula taeniata. Heredity,* 37: 253–269.

MURRAY, J. & CLARKE, B., 1980, The genus *Partula* on Moorea: speciation in progress. *Proceedings of the Royal Society of London,* B 211: 83–117.

MURRAY, J., JOHNSON, M. S. & CLARKE, B., 1982, Microhabitat differences among genetically similar species of *Partula. Evolution,* 36: 316–325.

PETRAS, M. L., 1967, Studies of natural populations of *Mus.* I. Biochemical polymorphisms and their bearing on breeding structure. *Evolution,* 21: 259–274.

PIMENTEL, D., SMITH, G. J. C. & SOANS, J., 1967, A population model of sympatric speciation. *American Naturalist,* 101: 493–504.

WRIGHT, S., 1946, Isolation by distance under diverse systems of mating. *Genetics,* 31: 39–59.

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SHELL POLYMORPHISM OF *THEBA PISANA*—THE EFFECTS OF RODENT DISTRIBUTION

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ABSTRACT

In *Theba pisana* of Israel, the relative frequency of effectively banded shells is positively associated with the extent of perennial cover: the more bushes in the habitat, the more frequent effectively banded shells are. This has been explained in terms of visual versus climatic selection. A white shell protects against radiation, whereas an effectively banded shell is more cryptic. In dense vegetation the snails are partly shaded from solar radiation, and hence can afford to be cryptic.

The Caesarea sands are characterized by their sharp transition between three vegetation associations: the *Ceratonia-Pistacia* association in which the extent of perennial cover is highest (and where we would expect to find the highest frequency of banded shells); the *Raetama-Helianthemum* association which is intermediate, and the *Artemisia* association where perennial cover is lowest (and where we would expect to find the lowest frequency of banded shells).

The results, however, are opposite to those expected: the highest frequency of effectively banded shells occurs in the *Artemisia* association, and the lowest in the *Ceratonia-Pistacia* association.

In the Caesarea sands, there is a very close association between the microdistribution of gerbils, which are predators of *T. pisana*, and the frequency of effectively banded shells. Variation in predation intensity could thus directly cause the variation in the distribution of cryptic morphs.

Our present work suggests that the high correlation found in Israel between the extent of perennial, bushy habitats on the one hand, and the relative frequency of cryptic morphs on the other, may result not only from the shading effects of vegetation, but because certain snail predators are present here and are not present amongst exposed habitats.

Key words: *Theba pisana*; shell polymorphism; morph distribution; rodent predation; climatic selection; visual selection.

INTRODUCTION AND RESEARCH AREA

Theba pisana (Müller) is a very abundant snail throughout the coastal plain of Israel, in Mediterranean habitats. A pattern of up to four pale bands decorates the shell, sometimes overlaid with numerous delicate, dark brown bandlets; the numbers of bands per shell are highly variable. The relative frequency of effectively banded shells (i.e. those with the upper, visible area of the shell banded) is positively associated with the extent of perennial cover: the more bushes in the habitat, the more frequent effectively banded shells are (Heller, 1981). There are two conflicting selective forces acting on this shell banding polymorphism: this diurnal species is exposed to solar radiation (especially in summer) and to predation by birds, mammals, and insects. A white shell protects the snail from radiation (by reflecting it), while banding is more cryptic and gives better protection against predators. In the very hot Israeli summer, a dark shell is adaptive only when visual selection is operative; otherwise, it is better to be white and avoid the danger of overheating. In the absence of visual selection we therefore expect to find almost monomorphic white populations in all habitats. (In winter, the ability to "warm up" could be an advantage to dark snails. Hence, perhaps even in Israel, not all snails would be white in the absence of predation). The effect of predation, as regards the shell banding polymorphism, will be more obvious in dense perennial vegetation (which partly protects the snails from radiation) than in exposed habitats consisting mainly of annuals that dry up in summer. Hence, a predominance of effectively unbanded morphs is found in exposed habitats, with a rising frequency of effectively banded morphs as the vegetation becomes denser (Heller, 1981).

However, dense vegetation is not necessarily associated with predation, and local differences in predation can cause deviations from the general picture just described. One case which exemplifies the role of predation in determining snail morph frequencies is presented in this paper. It focuses upon a selective factor that is frequently neglected in studies of shell polymorphism in landsnails (see reviews by Jones *et al.,* 1977, and Clarke *et al.,* 1978), i.e. the micro- and macrodistribution of snail predators.

The sandy soils near Caesarea, 35 km south of Haifa along the coastal plain, are characterized by a sharp transition between three vegetation associations: the *Artemisia monospermia* association on mobile sand-dunes; the *Raetama-Helianthemum stipulatum* association on stable sand; and the *Ceratonia-Pistacia* association on stable sand enriched by organic matter where this sand occurs above sandy loam (Kutiel, Danin & Orshan, 1980). The extent of vegetation cover is highest (90%) in the *Ceratonia-Pistacia* association; in *Raetama-Helianthemum* it is intermediate; and in the *Artemisia* association it is lowest (less than 40%). Since the frequency of effectively banded shells is, in general, positively related to the extent of shading vegetation, one would expect to find the highest frequency of effectively banded shells amongst *Ceratonia*; an intermediate situation in *Raetama*; and the lowest with *Artemisia.*

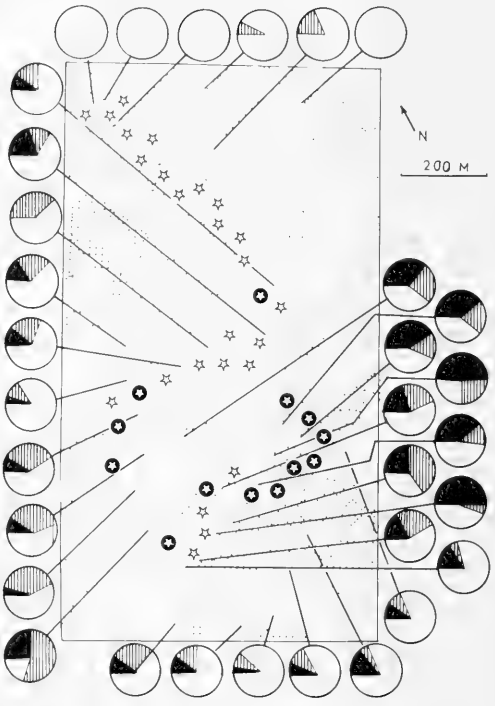

FIG. 1. Relative morph frequency of *T. pisana* in relation to the distribution of *Gerbillus allenbyi,* and to vegetation. *Theba*: Every sample is represented by a pie diagram, in which % 1234 is shown black, 0234 hatched, effectively not banded white. Gerbil: Each cluster of traps is represented by a star; in clusters where gerbils were trapped, the star has a black background. Vegetation: *Ceratonia-Pistacia* association is shown densely stippled; *Artemisia monospermia* association sparsely stippled; *Raetama-Helianthemum* association white.

RESULTS

The area of the Caesarea sands shown in Fig. 1 (Israel Grid 1435-2102) was sampled on 26 July 1979 in the following way: in each of the three habitats, ten samples were taken from the small area of one isolated bush. Separation between bushes is highest in the *Artemisia* habitat, and lowest in the *Ceratonia-Pistacia* habitat. All snails found in each such small area were collected and considered a sub-population. The relative frequency of each of the morphs was classified according to Heller (1981). The results are summarized in Table 1 and Fig. 1.

Within each of the three habitats there is a certain variation between samples, highest in *Artemisia* and *Raetama-Helianthemum*, and lowest in *Ceratonia-Pistacia* (Table 2).

Table 1 gives the overall mean morph frequency of each habitat. The differences between the habitats are greater than the differences within each of them, and are highly significant (χ^2 test, $\alpha << 0.001$). However, they are opposite to that expected: the highest frequency of effectively banded shells occurs in the sparsely covered *Artemisia* habitat, and the lowest frequency is amongst the densely covered *Ceratonia-Pistacia* habitat.

The area sampled is only 0.72 km^2. Differential microclimatic selection, if significant, should have favoured results opposite to what we actually found, since solar radiation must be stronger in the more exposed *Artemisia* habitat than in the dense *Ceratonia-Pistacia* one. In an Australian population of *T. pisana,* effectively banded snails move preferentially from open vegetation to sheltered habitats, for

TABLE 1. Actual values, and relative morph frequencies (in %) of *T. pisana* in the three habitats at the Caesarea sands.

Habitat	n	Effectively banded	0000	0034	0234	1234
Ceratonia-Pistacia	14	0	2	12	0	0
	28	0	4	24	0	0
	16	0	2	14	0	0
	3	0	2	1	0	0
	20	1	1	18	1	0
	8	0	2	6	0	0
	27	0	6	25	0	0
	9	2	2	5	2	0
	1	0	0	1	0	0
Total	126	3(2%)	21(17%)	102(81%)	3(2%)	0
Raetama-Helianthemum	27	23	1	3	16	7
	18	8	0	10	7	1
	11	5	1	5	4	1
	38	16	1	21	13	3
	24	4	5	15	3	1
	43	16	1	26	10	6
	19	6	1	12	4	2
	20	8	1	11	8	0
	35	12	1	22	5	7
	22	5	2	15	3	2
Total	257	103(40%)	14(5%)	140(54%)	73(28%)	30(12%)
Artemisia monospermia	35	22	0	13	12	10
	46	18	1	27	11	7
	16	8	0	8	2	6
	25	19	0	6	6	13
	14	9	0	5	3	6
	31	17	1	13	7	10
	23	14	0	9	8	6
	22	12	0	10	1	11
	24	10	2	12	7	3
	22	4	4	14	1	3
Total	258	133(52%)	8(3%)	117(45%)	58(22%)	75(29%)

TABLE 2. χ^2 test of heterogeneity between samples in each habitat. Only samples with more than 10 individuals are included.

Habitat	No. of samples	df	χ^2	α	Significance	Remarks
Ceratonia-Pistacia	5	4×1	2.67	<50	N.S.	Only the 0000 and 0034 categories have enough individuals
Raetama-Helianthemum	10	9×2	3.90	$< .01$	S	Only 0034, 0234 and 1234 have enough individuals
Artemisia monospermia	10	9×2	34.8	<0.01	S	Only the 0034, 0234 and 1234 have enough individuals

TABLE 3. Number of gerbils trapped at Caesarea.

Habitat	No. of gerbils caught	No. of gerbils trapped more than once	No. of clusters in which gerbils were trapped
Ceratonia-Pistacia	0	0	0
Raetama-Helianthemum	4	0	4
Artemisia monospermia	17	5	9

aestivation (Johnson, 1981). In the Caesarea sands, however, such preferential movements, if occurring at all, should again have favoured results opposite to what we actually found.

Remains of gnawed shells beneath bushes suggested that the rodent *Gerbillus allenbyi* Thomas might be one of the snail predators in this area. To examine the microdistribution of the gerbils, 120 mousetraps were set out for three consecutive nights (4 April–6 April 1980) in all three habitats. Within each habitat 13 trap clusters, each containing 3 traps, were set 10 to 20 m apart. The traps were set each evening at 1700 hr and examined the next morning at 0.500 hr. Trapped gerbils were marked and released. Each day we recorded the number of gerbils caught, the traps occupied, and also the extent of retrapping, so as to obtain a picture of the relative abundance of gerbils throughout the three habitats. The results, summarized in Table 3 and Fig. 1, suggest (from the high number of individuals trapped and from the high incidence of retrapping) that there is a permanent population of gerbils in the *Artemisia* habitat, with individuals wandering into *Raetama-Helianthemum* (where few individuals were trapped, no retrapping, and each trapping occurred at a different cluster); in the *Ceratonia-Pistacia* habitat there are apparently no gerbils at all. One *Rattus rattus* was trapped in the *Ceratonia-Pistacia* habitat.

G. allenbyi caught in the wild and kept in our laboratory readily ate *Theba* offered to them. Rats did not.

DISCUSSION

Banded shells are found on the Caesarea sands at a high frequency in the mobile sands sparsely covered by *Artemisia*; at a low frequency in the stable sands covered by the *Raetama-Helianthemum* association; and rarely on the stable sands enriched in organic matter and densely covered by *Ceratonia-Pistacia.* This parallels the relative abundance of the gerbil in this area. This parallelism between the microdistribution of the predator and the cryptic morph of the prey is close enough (Fig. 1) to suggest strongly, even though the evidence is only circumstantial, that it is indeed variation in the intensity of predation that directly causes the variation in distribution of the morphs. In a similar manner the song thrush, which is essentially a woodland species, predates *Cepaea nemoralis* much more in woodland than in nearby open habitats; hence visual selection in *C. nemoralis* is stronger in lowland wooded country than in downlands (Arnold, 1971).

The difference between morph frequencies in the *Artemisia* habitat and the *Ceratonia-Pistacia* habitat is considerable, dropping from about 52% effectively banded to 2% (via 40% in the *Raetama-Helianthemum* association) over the short distance of just 500 m. Apparently quite a strong selection differential is maintaining this cline: in the absence of such a differential, migration between habitats would abolish it very quickly. (We have records [Godot, 1981] that in Israel *T. pisana* may move at least 14 m within less than 40 days; in Australia, Johnson [1981] recorded a specimen moving 40 m).

Table 1 reveals quite considerable heterogeneity amongst samples within the *Artemisia* and *Raetama-Helianthemum* habitats, as compared with the relative homogeneity in the *Ceratonia-Pistacia* habitat. There is as yet no agreement, in the literature, as to the source of heterogeneity between relatively small landsnail populations that occur within a homogeneous habitat. One approach assumes a rather complicated array of modifiers operating on invisible ecological differences (Clarke,1966; Wolda, 1969). Another approach assumes the operation of genetic drift and the founder effect (Goodhart, 1962; Hickson, 1972). Both approaches assume that the populations observed, which at

present are too big to account for random processes, had past histories of "bottleneck" populations which were small enough to be affected by random processes.

A third approach can be adapted from Wright's models (1943, 1969) on the efficiency of selective and random processes between sub-populations in a homogeneous habitat. According to this approach, three parameters determine the amount of differentiation we can expect between sub-populations: the size of the selective differences (Δs); the size of the panmictic unit (N) (in this case, of the sub-population), and the amount of migration between the units (m).

When m is small we talk of a large population which is divided into relatively isolated sub-populations, and then we can expect much heterogeneity between them. The source of this heterogeneity depends upon N, the size of the sub-population: If it is small ($Nm < 50$), then the influence of genetic drift is much greater than selective forces. If N is big ($Nm > 50$), then the influence of selective forces is much more important. When m is big we talk of a large population that is more or less continuous, and the panmictic units are not isolated. In this case it is very hard to get much differentiation between sub-populations. If we do observe such differentiation, it is due to selective forces only when N is big, and it is mostly due to random processes when N is small. In the case of a continuous population, N is the "neighbourhood unit" (Wright, 1969), which is equivalent to the panmictic unit; and when $N < 1000$, then the possibility of random processes is very unlikely (Wright, 1943).

Finally, when referring to large populations, each composed of many sub-units (panmictic units or neighbourhood units), the differences between the large units are mostly selective, even when the sub-units themselves are very small. We can obtain considerable differences between the large units with even very small selective forces. When the sub-units are small and isolated, however, and therefore more exposed to random processes, the composed large population is subjected to more rapid evolution (by selective forces) than when m is large, and no differentiation is possible (Wright, 1943, 1969).

The hundreds of snails (a modest estimate) found in each of the vegetation habitats form populations that are far too large to allow for the morph distribution between the habitats to be explained in terms of random factors. However, we still have to explain the considerable heterogeneity found within the *Artemisia* and *Raetama-Helianthemum* habitats. There can be two reasons for this heterogeneity: 1) differential selection caused by slight differences in local predation pressures, resulting from the spatial distribution of the gerbils within each of the habitats, and 2) random processes, such as "bottleneck" populations and genetic drift.

On the basis of our observations alone, it is impossible to separate these two effects. It is noteworthy, however, that as the extent of snail migration correlates positively with the extent of vegetation cover (Gadot, 1981 and in preparation), migration between the bushes in the *Artemisia* habitat would be less than between similarly spaced bushes amongst the more densely covered habitat of *Ceratonia-Pistacia.* (And indeed, there is much more homogeneity in this latter habitat, as one would predict from our consideration of Wright's models.) Hence, even if spatial differences in selective pressure amongst different *Artemisia* bushes are slight, they will be reflected by corresponding differences in morph frequencies. In other habitats, however, there is less possibility for such fine differentiation.

We do not know why the gerbil does not enter the *Ceratonia-Pistacia* habitat. Perhaps gerbils are adapted to moving in open sands, and annual vegetation (which is very dense in the bushes and shrubs of this association) hinders their mobility. Also, since gerbils are burrowing animals, they may avoid sandy areas that lie upon harder soils, where digging is difficult. Gerbils are nocturnal, so it is mainly during the evening and night that they search for snails, selecting against the white, conspicuous shells on the basis of tone rather than colour (see Cain, 1953). In the dim, shadowy conditions of moonlight or starlight, the concealing effect of the bands makes the shells bearing them extremely cryptic (to the human eye), whereas the glossy, white, non-banded shells are very conspicuous. As gerbils seldom climb up bushes, their selective effect on the banding pattern of the snails would be stronger when the snail is near the ground—when copulating or laying eggs, for example—than when it is 1–2 m above the ground, where the snails usually aestivate.

One important outcome of this work is that the high correlation between the extent of perennial, bushy habitats on the one hand, and the relative frequency of cryptic morphs

of *T. pisana* on the other, may result not only from the shading effects of vegetation (Heller, 1981), but also because certain snail predators are present here and are not present in exposed habitats.

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REFERENCES CITED

ARNOLD, R., 1971, *Cepaea nemoralis* on the East Sussex South Downs, and the nature of area effects. *Heredity,* 26: 277–298.

CAIN, A. J., 1953, Visual selection by tone of *Cepaea nemoralis. Journal of Conchology,* 23: 333–336.

CLARKE, B., 1966, The evolution of morph-ratio clines. *American Naturalist,* 100: 389–402.

CLARKE, B., ARTHUR, W., HORSLEY, D. T. & PARKIN, D. T., 1978, Genetic variation and natural selection in pulmonate molluscs. In FRETTER, V. & PEAKE, J., ed., *Pulmonates.* Academic Press, London, 2A: 219–270.

GADOT, M., 1981, Interactions between two landsnail species in Israel, as regards their shell-banding polymorphism. Unpublished M.Sc. thesis, Hebrew University, Jerusalem. In Hebrew.

GOODHART, C., 1962, Variations in a colony of the snail *Cepaea nemoralis* (L.). *Journal of Animal Ecology,* 31: 207–237.

HELLER, J., 1981, Visual versus climatic selection of shell banding in the landsnail *Theba pisana* in Israel. *Journal of Zoology,* London, 194: 85–102.

HICKSON, T., 1972, A possible case of genetic drift in colonies of the land snail *Theba pisana. Heredity,* 45: 7–14.

JOHNSON, M., 1981, Effects of migration and habitat choice on shell banding frequencies in *Theba pisana* at a habitat boundary. *Heredity,* 47: 121–133.

JONES, J. S., LEITH, B. & RAWLINGS, P., 1977, Polymorphism in *Cepaea*: a problem with too many solutions? *Annual Review of Ecology and Systematics,* 8: 109–113.

KUTIEL, P., DANIN, A. & ORSHAN, G., 1980 ["1979/80"], Vegetation of the sandy soils near Caesarea, Israel. I. Plant communities, environment and succession. *Israel Journal of Botany,* 28: 20–35.

WOLDA, H., 1969, Fine distribution of morph frequencies in the snail *Cepaea nemoralis* near Gröningen. *Journal of Animal Ecology,* 38: 305–327.

WRIGHT, S., 1943, Isolation by distance. *Genetics,* 28: 114–138.

WRIGHT, S., 1969, *Evolution and the genetics of populations.* Vol. 2: *The theory of gene frequencies.* University of Chicago Press, Chicago and London, vii + 511 p.

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SHELL COLOURS OF DESERT LANDSNAILS

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ABSTRACT

White shells are associated with exposed habitats because they reflect more radiation than dark ones. Since deserts are exposed to severe solar radiation, one might expect to find a predominance of white snails in them. On the contrary, 20 out of 22 species in the Negev desert of Israel are not white. White snails in the Negev do occur occasionally, but as an exception, not a rule. Details of daytime resting sites (in the open and in shelter) for each species do not adequately account for this unexpected lack of white-shelled species.

Key words: shell colours; desert landsnails; climatic selection; visual selection; predation pressure.

INTRODUCTION

White shells reflect more radiation than dark ones. Therefore, snails in exposed resting sites with excess radiation are often subject to selection in favour of white shells. On a microgeographic scale, within restricted limits, white shells are therefore associated with exposed habitats in North America, western Europe, Australia, and Israel (Cain, 1977, and references therein; Johnson, 1980; Heller, 1981).

However, white shells are also very conspicuous against their natural background and therefore are at a selective disadvantage when visual selection is operative. In Mediterranean habitats (and as regards *Theba pisana*), a small colony of the snail-eating rodent *Gerbillus allenbyi* Thomas creates selective forces that override the effects of climatic selection (Heller & Gadot, this volume). In this paper, the question of climatic versus visual selection is extended to a more general scale: to that of shell colours in deserts.

Hot, arid deserts are almost devoid of vegetation and are exposed to much solar radiation (especially when compared with Mediterranean landscapes which are cooler and wetter, and therefore richer in shading vegetation). If climate is indeed of supreme selective importance in deserts, then one would expect to find in them a predominance of white-shelled species.

On the other hand, some deserts are also densely populated by rodents, many of which eat snails to balance their water regime. Certain desert-dwelling rodents such as *Gerbillus dasyurus* Wagner, *Acomys cahirinus* Desmarest, and *Elyomys melanurus* Wagner are well-known snail hunters; at one site in the Negev (Yom-Tov, 1970), an average of 184 gnawed shells were discovered in front of rodents' burrows. If visual rather than climatic selection is of supreme importance in deserts, then one would expect to find a predominance of non-white species.

Is there a predominance of white-shelled snails in deserts? This paper examines the question by surveying the shell colour of 23 of the 25 landsnail species found in the Negev desert, Israel.

RESEARCH AREA AND METHODS

The Negev is a hot, dry desert. In the northern and central Negev (the areas investigated here) average monthly maximum temperatures in summer reach 39°C and annual rainfall decreases from 250 mm in the north to 80 mm in the south. The 70 mm isohyet is the southernmost limit of Israel's malacofauna because beyond it rain is so scarce and irregular that only a few sparse populations can survive. Total annual insolation, averaging 186 K cal $cm^{-2}yr^{-1}$, is one of the most intense in the world (Stanhill, 1970). Because of the complex geo-lithologic composition of the Negev, the landscape and soils are heterogeneous, ranging from bare rock outcrops and desert lithosols to coarse desert alluvium, sand dunes, and loess. Further physical and botanical details on the Negev can be found in the Atlas of Israel (1956) and Danin (1970).

Throughout most of Israel's Mediterranean region the soils are darker than in the Negev: about 80% of the mountain soils consist of reddish brown terra-rossa, formed upon limestone, and about 16% consist of pale greyish rendzina, formed upon chalk (where, incidentally, the snail fauna is far less diverse; soil data are from the Atlas of Israel (1956). Also in the plains, most soils are dark, whether the greyish-black soil left by past swamps or the dark brown soil eroded from the mountains. Yellow sands are pale, of course, but they form only a narrow belt 2–5 km wide along the Mediterranean coast. In contrast, throughout the Negev, practically all soils are in a dirty pale yellow range, whether formed by disintegrating limestone or chalk, wind-borne loess, or water-borne sand which here covers vast areas. Only flint stone occurring occasionally, whether as hammada or outcrops amongst the white chalk could be classified as a dark component in the desert environment.

These differing background colours between the Mediterranean and desert regions create a certain methodological problem. If visual selection were operative in the desert it would produce pale yellow shells (especially in those parts of the desert where there is little vegetation, and the bare soil forms the main background of those snails moving from one shrub, or crevice, to another). However, pale shells are also what one might expect if climatic selection were operating.

To separate the effects of visual from climatic selection the shells in this study are classified when viewed from above as either white or otherwise. In the helicellines, where shell colour may change with age, only the ultimate whorl was scored. White shells are conspicuous on any soil (including the pale yellow of deserts), both during the day and night, and would be disadvantaged by visual selection; however, they are the optimum colour of any snail subject to strong insolation, and would therefore be favoured in deserts if climatic selection in deserts is indeed of major, overriding importance.

This classification of "white or otherwise" is, of course, oversimplified. White can be only white, whereas a non-white shell can range from dark to almost white. *Levantina hierosolyma*, for instance, has a five-banded shell in which white flecks partly cover the bands, and thus is whiter than *Helix engaddensis* which has five similar bands without white flecks. Nevertheless, for the purpose of separating the effects of visual from those of climatic selection, this classification will probably suffice.

I collected the snails on various field surveys since 1968. To avoid erroneously recording empty, sun-bleached shells as white ones, special care was taken to score only those snails collected alive. The systematics follows Rochanaburananda (1968), Heller (1975), Forcart (1976) and others. All specimens are deposited in the mollusc collection of the Zoological Museum of the Hebrew University of Jerusalem, Israel (HUJ).

RESULTS AND DISCUSSION

Table 1 summarizes the results. Of 22 species (belonging to 10 genera and 8 families) only three—*Sphincterochila zonata, S. prophetarum* and *Xerocrassa seetzenii*—are white. By comparison, in France 4 out of 77 Helicacea species are white (Cain, 1977).

Sphincterochila, one of the most widespread genera in the Negev, inhabits stony hill slopes, loess plateaus, hammadas and sands. Its two desert species, *S. zonata* and *S. prophetarum,* are indeed white-shelled, and very conspicuous on almost every natural background. By experimentally painting *S. zonata* shells black, Schmidt-Nielsen *et al.* (1971) caused a lethal rise in body temperatures. When considering only these two species, *Sphincterochila* may indeed seem to be a classical case for white shells in deserts. A broader look at the genus shows, however, that *Sphincterochila* is found also in Mediterranean landscapes, where it is represented by two species: *S. cariosa* (Olivier) (in mountains) and *S. aharoni* (Kobelt) (in the coastal plain). Another species, *S. fimbriata* (Bourguignat), lives mainly in the semi-arid zone between the Mediterranean area and the desert. Just like the desert species, these Mediterranean species are all pure white. *Sphincterochila* is thus monomorphically white throughout its range of habitats in Israel, whether in dry exposed desert or dense Mediterranean vegetation.

Xerocrassa is also a widespread genus inhabiting sands, loess and rocky hill slopes. It is the desert equivalent of the closely related, highly polymorphic *Xeropicta* (which inhabits Mediterranean habitats). In *Xeropicta* white, non-banded shells along the coastal plain of Israel are more frequent in exposed than in sheltered habitats (Heller & Volokita,

TABLE 1. Shell colour of landsnails from the Negev desert. Species that spend most of their inactive time beneath stones and in crevices are marked with an asterisk (*).

Species	Colour
Pupillidae	
**Pupoides coenopictus* Hutton	uniformly pale horny brown
Chondrinae	
**Granopupa granum* Draparnaud	uniformly pale horny brown, sometimes blackish
Enidae	
**Buliminus therinus* Bourguignat	uniformly pale horny brown
**B. alepensis* Pfeiffer	uniformly pale horny brown
**B. negevensis* Heller	uniformly pale horny brown
**B. lamprostatus* Bourguignat	uniformly pale horny brown
**B. sinaiensis* Heller	uniformly pale horny brown
**B. glabratus* Mousson	uniformly pale horny brown
Euchondrus desertorum Rochanaburananda	
**E. albulus* Mousson	uniformly pale horny brown, ultimate whorl is often white
Sphincterochilidae	
Sphincterochila zonata Bourguignat	white
**S. prophetarum* Bourguignat	white
Helicellidae	
Xerocrassa seetzenii Pfeiffer	shell bands: polymorphic, mainly white
X. erkelii Kobelt	shell bands: polymorphic, mainly banded; seldom white
X. pilsbryi Forcart	shell bands: polymorphic, mainly banded
X. helleri Forcart	shell bands: polymorphic, mainly horny brown
**X. langloisiana* Bourguignat	polymorphic, pale brown or white; mainly brown
**X. pseudojacosta* Forcart	uniformly pale horny brown
**X. tuberculosa* Conrad	uniformly pale horny brown
Helicigonidae	
**Caracolina lenticula* Michaud	uniformly horny brown
Helicidae	
**Eremina desertorum* Forskal	shell bands: monomorphic, four brown bands
**Levantina hierosolyma* Mousson	shell bands: monomorphic, five brown bands on brown-mottled shell
**Helix engaddensis* Bourguignat	shell bands: monomorphic, five brown bands on brown shell

1981). Hence, one might also expect *Xerocrassa* to be predominantly white, since it occupies desert habitats that are far more exposed than those of *Xeropicta*. In one species, *X. seetzenii,* predominantly white populations are indeed found. *X. seetzenii* occupies the northern parts of the Negev where the climate is rainier (200 mm) than in the southern parts (70 mm). Farther south, where the climate is more eremic (and where one would, therefore, expect *Xerocrassa* populations to have a higher proportion of white shells than *X. seetzenii*), the three species replacing *X. seetzenii* (*X. pilsbryi, X. erkelii* and *X. helleri*) are dark, sometimes heavily pigmented, and not one of the samples contains more than 25% white shells (Fig. 1). Nevo, Bar-El & Bar (1981) found that in *Xerocrassa* the proportion of white shells increased southwards from 21% in the Qarantal region to 59% in the Negev. However, their cline is based only upon three to four samples. In the present work, where 21 samples (each consisting of 20 to 35 snails) were examined in the Negev alone, no southwards increase in the proportion of white shells was found. Other *Xerocrassa* species, such as *X. pseudojacosta* and *X. tuberculosa,* are uniformly pale brown. *X. langloisiana,* usually pale dirty brown, also has a white morph. Only 15% of the sample from Arad is white. By comparison, in Degania, which is 160 km north of Arad in the Mediterranean region, over 80% of a sample in the HUJ museum consists of white shells. Thus *X. langloisiana* also does not seem to support the "white

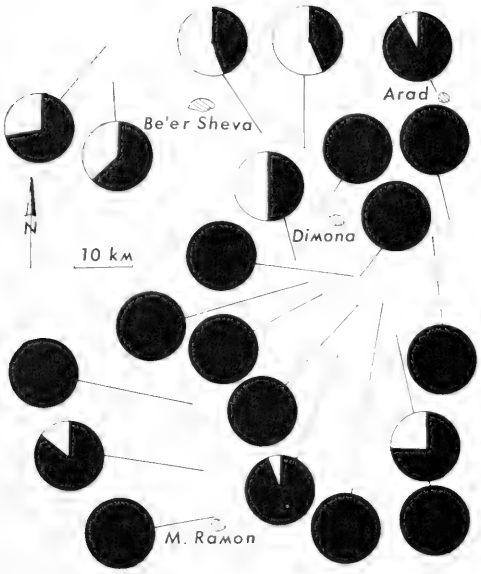

FIG. 1. Relative frequency of white shells (white areas in pie diagrams), in populations of *Xerocrassa* spp. from the Negev. *X. seetzenii, X. pilsbryi, X. erkelii* and *X. helleri* form a complex of parapatric species that are all morphologically close to one another, as compared to other *Xerocrassa* species found in this survey.

shells in deserts" theory, but more samples are needed.

Eremina desertorum is of special interest to the problems raised in this study because it lives on bushes in the sands of Mishor Yamin and Mishor Rotem; in a way it is the desert equivalent of the highly polymorphic *Theba pisana* (Müller) which also inhabits bushes in sandy habitats, but in Mediterranean rather than desert landscapes. Since in *Theba pisana* white, non-banded shells are more frequent in exposed than in sheltered habitats (Heller, 1981) one might also expect most *E. desertorum* shells to be white. On the contrary, all the shells found possess four broad, rather dark bands. *Eremina* in Israel is thus a fully-banded monomorph and refutes the "white shells in deserts" theory. Evidence has been given elsewhere (Heller, 1981) that in *Theba pisana* in Israel the proportion of effectively white shells does not increase southwards where the species encounters more arid conditions.

A detailed habitat classification of the Negev species into distinct ecological locations (deep crevices, beneath stones, on the ground, or upon bushes) would have been very desirable for the purposes of this study, but unfortunately there are gaps in collection data; therefore, I shall divide them into only two broad categories: those that spend most of their inactive resting time mainly beneath stones and in crevices, and those that are found also or exclusively elsewhere, whether on the ground or in bushes. In deserts, as in Mediterranean habitats, crevice dwellers need not be white if they come out only at night or on those few mildly warm days that follow rare winter rains.

As Table 1 shows, the majority of Negev species are crevice dwellers; only four or five species are not (*Sphincterochila zonata, Xerocrassa seetzenii, X. erkelii, X. pilsbryi* and perhaps *X. helleri,* for which there are too few data). By comparison, in the Mediterranean region most of the species are also crevice dwellers, and only five (*Theba pisana, Xeropicta vestalis* (Pfeiffer), *Monacha haifaensis* (Pallary), *Sphincterochila cariosa,* and *S. aharoni*) are not. Those Negev species that are found beneath stones and also above ground include both white species, such as *S. zonata* and *X. seetzenii,* and also non-white ones, such as *Eremina desertorum* and *X. pilsbryi.* Of the three white Negev species *Sphincterochila prophetarum* is found mainly beneath stones (not the habitat that one might expect of a white snail). Another, *S. zonata,* dwells mainly upon the ground and in bushes; it is predated by rodents rarely because of the extreme thickness of its shell (Yom-Tov, 1970). The third species, *Xerocrassa seetzenii,* is mainly a bush dweller; many of its populations come from localities consisting of loess, soft chalk, or other substrata that the snail-eating rodents *Acomys, Eliomys,* and *Gerbillus dasyurus* (being obligatory rock-dwellers) do not occupy.

In many crevice-dwelling species the uniformly horny-brown colour of the shell is pale when compared to European crevice-dwelling species. Because the climate in Europe is much colder, one is tempted to believe this is evidence in favour of climatic selection. Here, however, the pale yellow colour of the soil also must be considered. From the viewpoint of climatic selection, a pale shell has little benefit for a nocturnal animal which spends its days in crevices and beneath stones, and emerges only at night. From the viewpoint of visual selection, however, even nocturnal animals would benefit from closer matching with the light tones of their environment.

As mentioned in the Introduction, many snails that habitually rest on surfaces exposed to the sun have white shells. It might be wrong, however, to infer from this that deserts, having shadeless habitats with high insolation consequently have a high frequency of white-shelled species. This theory is both reasonable and appealing, but in the Negev at least there is no justification for it. Apparently even in deserts, where solar radiation is exceptionally high, selective pressures for anti-radiation devices can be overridden by other, greater selective forces. One such force could be predation by rodents. This work (together with that of Yom-Tov, 1970, and Heller & Gadot, this volume) suggests that many desert snails can be white only in the absence of heavy visual predation.

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REFERENCES CITED

ATLAS OF ISRAEL, 1956, Publ. Dept. of Surveys and Bialik Inst., Jerusalem. In Hebrew, 120 p.

CAIN, A. J., 1977, The uniqueness of the polymorphism of *Cepaea* (Pulmonata: Helicidae) in western Europe. *Journal of Conchology,* 29: 129–136.

DANIN, A., 1970, *A phytosociological-ecological study of the northern Negev of Israel.* Unpublished Ph.D. thesis, Hebrew Univ., Jerusalem, 230 p. In Hebrew, English summary.

FORCART, L., 1976, Die Cochlicellinae und Helicellinae von Palästina und Sinai. *Archiv für Molluskenkunde,* 106: 123–189.

HELLER, J., 1975, The taxonomy, distribution and faunal succession of *Buliminus* (Pulmonata: Enidae) in Israel. *Zoological Journal of the Linnean Society,* 57: 1–57.

HELLER, J., 1981, Visual versus climatic selection of shell banding in the landsnail *Theba pisana* in Israel. *Journal of Zoology, London,* 194: 85–101.

HELLER, J. & GADOT, M., 1984, Shell polymorphism of *Theba pisana*—the effects of rodent distribution. *Malacologia,* 25: 249–254.

HELLER, J. & VOLOKITA, M., 1981, Shell-banding polymorphism of the land snail *Xeropicta vestalis* along the coastal plain of Israel. *Biological Journal of the Linnean Society,* 16: 279–284.

JOHNSON, M., 1980, Association of shell banding and habitat in a colony of the landsnail *Theba pisana. Heredity,* 45: 7–14.

NEVO, E., BAR-EL, C. & BAR, Z., 1981, Genetic structure and climatic correlates of desert landsnails. *Oecologia,* 48: 199–208.

ROCHANABURANANDA, T., 1968, Anatomical investigations of the genus *Jaminia* from Israel. Unpublished M. Sc. thesis, Hebrew Univ. Jerusalem, 47 p.

SCHMIDT-NIELSEN, K., TAYLOR, C. R. & SHKOLNIK, A., 1971, Desert snails: problems of heat, water and food. *Journal of Experimental Biology,* 55: 385–398.

STANHILL, G., 1970, Measurements of global solar radiation in Israel. *Israel Journal of Earth-Sciences,* 19: 91–96.

YOM-TOV, Y., 1970, The effect of predation on population densities of some desert snails. *Ecology,* 51: 907–911.

MALACOLOGIA, 1984, 25(2): 361–380

ECOGENETICS OF *THEBA PISANA* (PULMONATA: HELICIDAE) AT THE NORTHERN EDGE OF ITS RANGE

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ABSTRACT

The highly variable coastal Mediterranean land snail *Theba pisana* reaches the northern limit of its range at a small number of localities in the southern and western parts of the British Isles, where its local distribution indicates the importance of climatic factors. At Tenby (South Wales) the life-cycle is biennial, not annual, in response to the relatively short growing season, and shells tend to be darker than in Mediterranean localities.

The inheritance of shell banding pattern at Tenby is shown to be controlled by three loci (at least) with two alleles showing dominance at each. Epistasy allows only four main phenotypes to appear: plain unbanded, dotty unbanded, dark five-banded, yellow five-banded. The characterisation of these morphs has allowed their frequencies in the field at Tenby to be determined. Morph frequencies differ at the different sites, but appear fairly constant over five years, despite the advantage banded snails appear to have during the second year of the life-cycle. Reasons for this apparent contradiction are discussed. The selective agent cannot be identified, but drift is unable to account for the trend.

Brief comparisons of this polymorphism with that exhibited elsewhere, suggest three geographical regions (Britain, northern France, the Mediterranean), each with a different range of variation.

INTRODUCTION

Theba pisana (Müller) is locally abundant in coastal habitats around almost the whole of the Mediterranean area. Its range extends southwards along the Atlantic coast of North Africa as far as northern Mauritania, and northwards up the Iberian and French coasts, reaching its continental mainland limit on the Belgian coast, the northernmost colonies in Belgium being considered by Deblock & Hoestlandt (1967) to be the result of artificial introductions. There are colonies in the Channel Islands, in the south-west of England and Wales, and on the east coast of Ireland, this being its most northerly locality. Such marginal populations will often be subject to more extreme environmental conditions, and are often more isolated from each other than are more central populations. Ecological and genetic differences related to these factors would therefore be expected.

The local distribution of *T. pisana* at Tenby, South Wales (the largest colony in mainland Britain), on only south- and west-facing slopes in areas where north- and east-facing slopes appear suitable, indicates the importance that climatic factors may have in determining the northern limits of its distribution (Cowie, 1982).

Changes in population size-structure over four years, with estimates of growth rates and observations of reproductive maturity, show that *T. pisana* is biennial and semelparous at Tenby (Cowie, 1984). Populations elsewhere in the species' range adopt both biennial and annual patterns, depending on the length of time they are forced into aestivation by the hot, dry Mediterranean summers, or into hibernation by the cool north Atlantic winters (Sacchi, 1971, 1977, 1978; Sacchi & Violani, 1977; Heller, 1982, and references therein; Cowie, 1984).

T. pisana is renowned for its immense range of variation in shell pattern. Previous workers have failed to develop an adequate classification of this variation; for instance, Lamarck virtually gave up trying to describe the variation succinctly (Cain, 1981); Germain (1929) concluded that all intermediates could

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be found between the numerous varieties then named; and neither Johnson (1980) nor Heller (1981) were able to do more than classify their shells into three and five broad categories respectively. These difficulties arose largely because of the absence of any knowledge of the genetic control of the variation. Heller (1981) quoted unpublished work by G. Lewis which indicated that an allele producing an unbanded shell was dominant to an allele for banding, but nothing further has been published regarding the genetics of shell pattern in *T. pisana.*

The purpose of this paper is first to present the results of a series of breeding experiments using specimens of *T. pisana* from the Tenby colony; with the paper by Cain (1984, this volume), this is the first information, obtained from breeding experiments, to become available on the genetics of shell pattern in *T. pisana.* With this as a basis, variation in the field at Tenby is investigated in some detail, and compared briefly with variation seen elsewhere in Britain, northern France and the Mediterranean region.

BREEDING EXPERIMENTS AND THE GENETICS OF THE VARIATION AT TENBY

Methods

The original stock of breeding animals was derived from the Tenby colony. Only small juveniles were used for breeding since it is known that snails as small as 10 mm diameter may be able to mate and breed successfully (Cowie, 1980). Further, only rapidly-growing individuals were used. These precautions ensured the use of only healthy, virgin snails. Each pair was set up in a plastic lunch-box (17.5 × 11.5 × 6 cm) with a layer of soil about 4 cm in depth. An area of about 7 × 8 cm was cut at one end of the lid of each box and covered with mesh netting material to allow ventilation. The boxes were kept in a room in which the temperature varied from about 10°C at night during cold spells in winter, to about 21°C during the day in summer. There was a small diurnal variation in temperature. Day length was not altered artificially, except that during the winter, artificial lights were kept on most weekdays until about 1700 or 1800 hr. The soil in the boxes was kept moist, and the animals were fed twice a week on a ground-up mixture of one part calcium carbonate, one part oat breakfast cereal ('Ready Brek') and one part dried skimmed milk ('Marvel'). This mixture was sprinkled onto the dampened solid part of the box lid. This food appeared ample, and on this regime *T. pisana* has been regularly grown to full adult size in much less than a year in the laboratory. When juveniles appeared, they were transferred into a sufficient number of boxes to prevent gross overcrowding, but despite this, some animals became stunted, or growth was uneven, which in some cases made classification difficult. However, most shells could be scored.

Description of banding morphs

Shells of *T. pisana,* like those of most helicid species, are pentataeniate, as indicated by Taylor (1912). The positions of the bands are shown in Fig. 1, in which they are numbered in the standard fashion, starting with band 1 nearest the suture. Heller (1981) has argued that *T. pisana* is in fact tetrataeniate; it is not clear from his picture whether his shells do not possess band 1, or their bands 1 and 2 are fused. As yet, there seems to be no good reason to depart from the pentataeniate model used in the descriptions of most helicids so far studied.

Although it is possible to use a simple banding formula (as described for *Cepaea* by Murray, 1975), this can not describe adequately the complex variation in *T. pisana.* The multitude of varietal names used within the species (Taylor, 1912) were given in ignorance of the genetics of the variation, and so a new series of vernacular names plus modified formulae which reflect the genetic basis of the variation are introduced here and by Cain (this volume).

The differentiation of the Tenby banding morphs rests heavily upon the results of the breeding experiments described below. However, for the sake of clarity, the appearance of each morph is described at this stage, and a selection of each is shown in Fig. 2. The major division is between unbanded and five-banded shells; these two groups are then subdivided. In all groups, if growth is halted (for instance by overcrowding in the breeding boxes) but subsequently restarts, the pattern may appear to change abruptly. In all such cases, the shells were scored as belonging to the pattern before the change, since this was

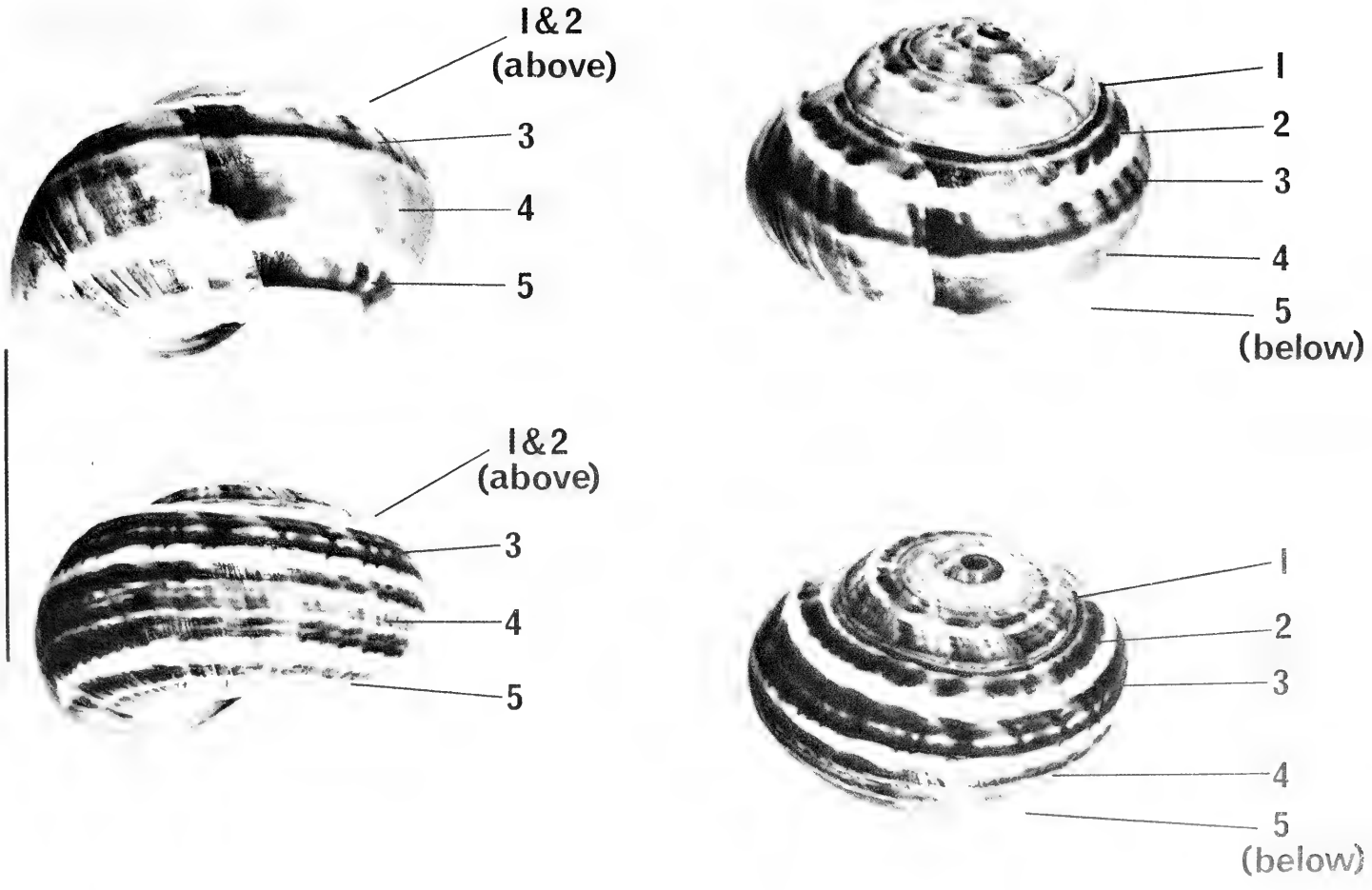

FIG. 1. Five-banded shells with the bands numbered in the standard fashion. On this and all other figures of shells, the bar represents 1 cm.

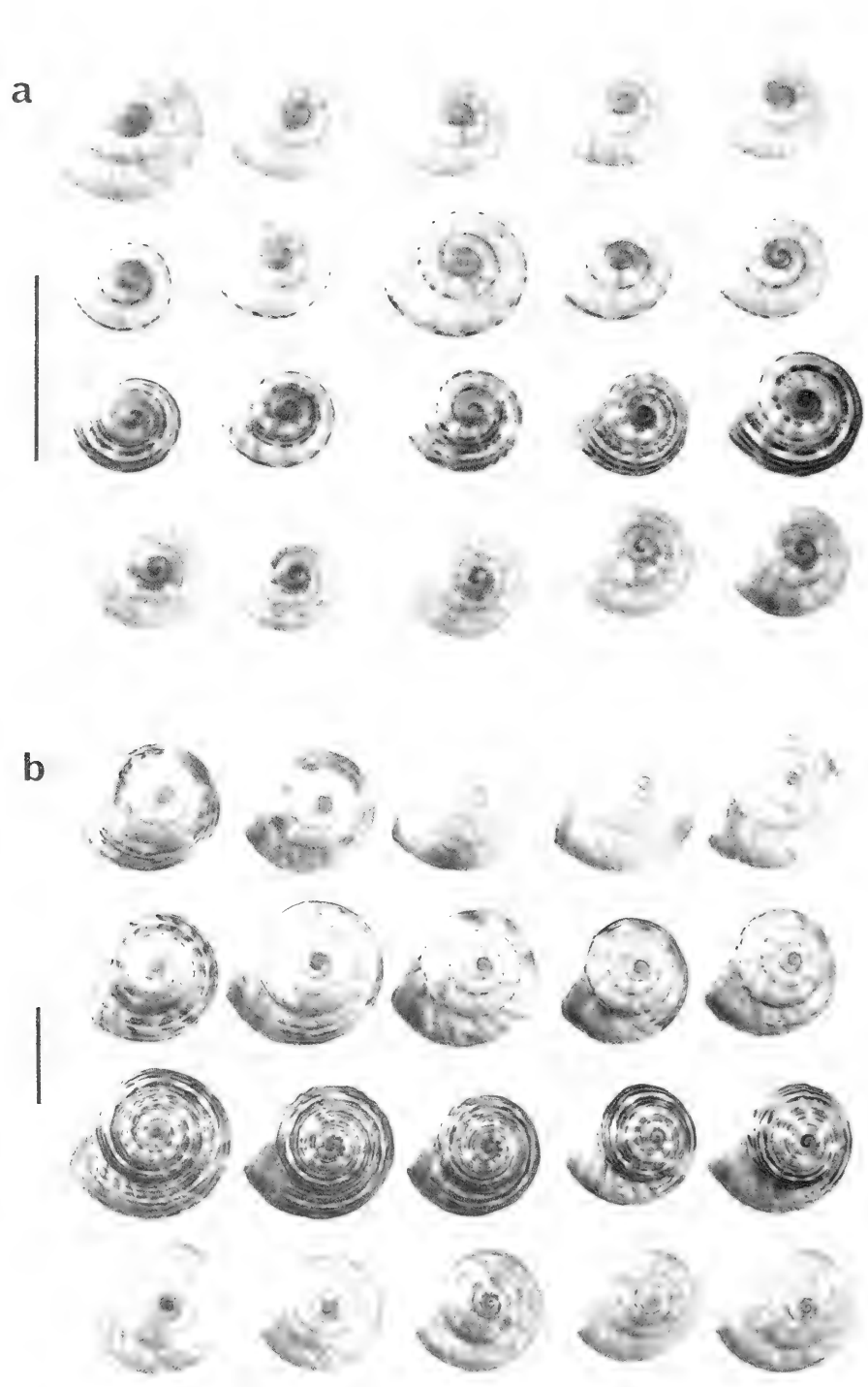

FIG. 2. The four main morphs at Tenby. a—juveniles, b—adults. In both a and b: top row—plain 00000, second row—dotty 00000, third row—dark 12345, bottom row—yellow 12345.

the only way in which the results of the breeding experiment could be adequately explained.

Unbanded:—(a) Plain 00000—The early whorls lack bands, but virtually all shells acquire some banding on later whorls. Lower bands, especially band 5, may begin earlier than the upper ones. There is much variation in the timing of the start of these bands (see Fig. 2). (To avoid the introduction of an overcomplex nomenclature, these shells are scored as unbanded, although they may be different genetically from completely unbanded shells. This is discussed further below, and by Cain, this volume.)

(b) Dotty 00000—In all respects similar to plain, except for the presence of a row of dots along, or just above, the keel of the shell, i.e. at about the position of the lower edge of band 3. This form, therefore, does not completely lack banding pigment on the upper whorls, but in order to interpret the results of the breeding programme it is necessary to consider both plain and dotty individuals as unbanded. Shells with the faintest hint of dots have been scored as dotty.

Five-banded:—(a) Dark 12345—All bands are present. Each is more or less broken up longitudinally into fine, dark brown lines, which may themselves be broken transversely into dots and dashes, augmented with feathering along their edges, or fused to varying extents producing arrow-head shapes and blocks. This lineolation appears to be superimposed upon a yellowish-buff background band-colour, darker than the remainder of the shell. In most shells, dark lineolations are present from the first whorl on the upper bands (1 and 2 particularly) and on band 5. Band 4 is the last to acquire them (usually during the third whorl).

(b) Yellow 12345—All bands are present, but only as the yellowish-buff background stripe. The bands are usually broken up transversely to some extent, and thus appear as a sequence of rather ill-defined blocks. No dark lineolations are superimposed, although there may be a hint of them in some shells, on both earlier and later whorls.

(c) Intermediate forms—It is not clear whether any of these strictly constitutes a morph, or whether the variation is under polygenic control; this is discussed further below. The bands may be completely lineolate, but with the lineolations barely darker than the yellowish background stripe. Many intermediates between this and the dark lineolate form (dark 12345) occur. Alternatively, there may be very few lineolations, yet these are very dark, again with many intermediates between this and the dark, fully lineolate form.

Genetic relationships between banding forms

The results of all the matings are presented in Table 1. They can only be understood in terms of a minimum of three loci:

Unbanded/five-banded:—Combining all types of unbanded shells in one class, and all banded shells in the other, matings TP 152, 156 and 180 (unbanded × unbanded) each show a 3:1 ratio of unbanded to banded progeny. Matings TPR 212 and F 9 (unbanded × banded) gave only unbanded progeny. Clearly, unbanded is dominant to five-banded. The segregation is confirmed by matings TPR 213, F 11, 15, 16, 20 and F 29 (unbanded × banded) which gave 1:1 ratios. No banded × banded mating gave any unbanded progeny. No matings are difficult to interpret.

Plain/dotty:—The 3:1 segregations in the progeny of matings TP 155, 171, 172 and 178 indicate that plain is dominant to dotty. The segregation is confirmed by 1:1 ratios in the progeny of TP 160 and F 9, and by both 3:1 and 1:1 ratios within the unbanded class of further matings in which other morphs appear: TP 152 (3:1), 156 (3:1), 180 (1:1), F 16 (1:1), 19 (1:1) and 29 (1:1). The progeny of matings F 11, 15 and 20 show the segregation but the numbers are too low to be sure of the ratio.

Since plain, dotty and banded individuals appear together in the progeny of these matings, a minimum of two loci must be involved. If plain and dotty are allelic, and banded is at a second locus, then the results of matings TP 152 and 156 can be explained as 9:3:4 ratios of plain:dotty:banded (i.e. 3:1 unbanded to banded, and 3:1 plain to dotty within the unbandeds); TP 180 as a 3:3:2 ratio (i.e. 3:1 unbanded to banded, and 1:1 plain to dotty within the unbandeds); F 16 and 19 as 1:1:2 ratios (i.e. 1:1 unbanded to banded, and 1:1 plain to dotty within the unbandeds). F 11, 15, 20 and 29 are similar but

TABLE 1. Results of breeding experiments—banding pattern.

Mating	Parental phenotypes	Progeny phenotypes					
		Unbanded		Banded			
		Plain	Dotty	Dark	Intermed.	Yellow	Total
TP152	plain × plain	16	3	8			27
TP154	yellow × yellow			81		39	120
TP155	plain × plain	24	2				26
TP156	?dotty × dotty	93	41	44			178
TP160	plain × dotty	188	159				347
TP169	yellow × yellow					154	154
TP171	plain × plain	77	29				106
TP172	plain × plain	12	4				16
TP178	plain × plain	26	6				32
TP180	plain × plain	35	50	28			113
TP183	dark × intermed.			75			75
TPR212	dotty × yellow	43	5				48
TPR213	dotty × yellow	23	5		8	13	49
TPR216	dark × dark			122			122
TPR217	dark × dark			11			11
TPR218	dark × dark			25	16	1	42
TPR220	dark × dark			77			77
TPR221	dark × yellow			10	7	16	33
TPR227	dark × dark			38			38
F1	intermed. × yellow			4	53	47	104
F5	dark × intermed.			16	11	2	29
F9	plain × dark	101	105				206
F11	plain × dark	4	5	4	4		17
F15	plain × dark	4	2	1	2		9
F16	dotty × yellow	36	35	22	14	23	130
F19	plain × dark	23	25	16	23	9	96
F20	plain × dark	5	5	3	1	3	17
F24	dark × intermed.			7	19	18	44
F26	intermed. × yellow				30	31	61
F28	dark × dark			4			4
F29	dotty × dark	22	14	10	8	4	58
F30	dark × dark			28			28

with rather few progeny. In none of these matings is the effect of the dotty allele clearly exhibited in the banded progeny and it has not been scored in them. Cain (this volume) discusses the expression of dotty in bandeds more fully.

In contrast to these clear-cut results, four matings present difficulties of interpretation. Mating TP 156 is of a clearly dotty individual mated with one in which it is difficult to be sure of the presence of dots, these perhaps lie obscured in the suture. Despite one or both these parents being dotty, the progeny show a 3:1 ratio of plain to dotty. Since, therefore, both parents must have been heterozygous plain/dotty, the simplest hypothesis explaining this result is that there must be penetrance of the dotty character such that it is expressed in a heterozygote. This hypothesis can also be used to explain the results of matings TPR 212 and 213 (both dotty × banded). In these, either (i) there is penetrance of the dotty allele in the unbanded parents (and both were heterozygous plain/dotty), combined with a low number of dotty individuals (especially in TPR 212) in a 3:1 ratio within the unbanded progeny; or (ii) the unbanded adult was in fact homozygous dotty and the banded parent homozygous plain, and there was penetrance of the dotty allele in some of the progeny, which must all, if this is the case, be heterozygous plain/dotty. Either way, there must be penetrance of the recessive character of heterozygotes (equivalent to incomplete dominance of plain). Since there is no clear bias towards plain in the numbers

of progeny in the majority of these matings, either (i) penetrance of the dotty allele of heterozygotes does not occur in all matings, or (ii) non-expression of dotty in some dotty homozygotes acts to reverse any such trend, and to make its detection more difficult; certainly the dots can be extremely faint and it may only be a small step to not being expressed at all. Indeed, the simplest explanation of the fourth difficult mating, TP 180, is that one parent was homozygous dotty but failed to expresss the dotty phenotype; the other parent would then have been heterozygous plain/dotty, thus giving the 1:1 ratio within the unbanded progeny. These rather complex explanations can only be considered at this stage as hypotheses, and not necessarily as the correct interpretations of these difficult matings. Other genes could be acting as suppressors, or to reverse dominance relationships. The data are insufficient to comment further on this.

Dark/yellow:—This is the most difficult locus to interpret because of the occurrence in some matings of forms intermediate between dark and yellow. However, within other matings the banded progeny are very similar to each other, and are either yellow (TP 169) or dark (TP 152, 156, 180, 183, TPR 216, 217, 220, 227, F 28 and F 30). In no mating do the banded progeny all belong to a distinct intermediate group. The two extremes of the range of banding are thus defined by the above matings and can be considered as morphs. In only one mating (TP 154) is there a clear-cut segregation: the 3:1 ratio of dark to a yellow form (both varying little) indicates that dark is dominant. However, the yellow progeny, although varying little, show hardly any overlap with the yellow form of TP 169 (which defines the yellow extreme of banding), the latter being slightly paler and with less indication of lineolation. This suggests a further morph. An unexplained complication is that both parents of TP 154 belong to the yellow form in their progeny and not to the dark form, which, however, on the numbers is dominant. Irregular penetrance or dominance failure may again be involved.

The remaining matings all gave some intermediate banded progeny. In no case was there a distinct division between these and the dark or yellow progeny, either or both of which were present in all of these matings. However, there is still an indication of some form of genetic control. The matings which gave only yellow and intermediate (no dark) progeny (TPR 213, F 26) or very few dark progeny (F 1) had one yellow parent. Both those matings lacking yellow progeny (F 11, 15), although with low numbers, and those with only few yellow progeny (TPR 218, F 5, 29) have at least one dark parent. Those with a more even spread (F 16, 19, 24) have either dark or yellow. F 20 has too few progeny to allow comment. It appears that a number of banding-modifier alleles may be involved, at a different locus (or loci) from the dark/yellow locus; or a number of alleles at the same locus, but with a range of dominance.

Matings F 16 and 19 show a 1:1 segregation of unbanded: five-banded, and both dark and yellow forms appear within the banded class. Since the banded parent of F 16 was yellow, and that of F 19 dark, these results are incompatible with unbanded, dark and yellow all being at the same locus, although this assumes no penetrance irregularities. Furthermore, since plain and dotty also segregate within the unbanded progeny of these matings, they cannot be at the same locus as dark and yellow. Indeed, matings F 16, 19, 20 and 29 (all unbanded × banded) gave all four classes of progeny and can only be explained in terms of a minimum of three loci, with other possible loci modifying the five-banded class further. It is not possible to detect linkage, since unbanded is epistatic to dark and yellow, and five-banded is epistatic to plain and dotty.

The results of five of the thirty-two matings described here have been suggested as being explained by penetrance of a recessive allele in heterozygotes (TP 154—dark/yellow, and TP 156, TPR 212, 213—all plain/dotty), or by lack of expression of a recessive allele even when homozygous (TP 180—dotty). This can only be considered as a hypothesis at this stage; the results could also be explained by more complex ideas, for instance, multiple alleles with different dominances. Variable penetrance and expressivity are rare, but not unknown in *Cepaea* (Cain, King & Sheppard, 1960; Wolda, 1969; A. J. Cain, personal communication). Their occurrence in *T. pisana* may be more extensive than this study has suggested, since penetrance of a recessive allele in heterozygotes, combined with its non-expression in homozygotes, could result in no radical departures from expected ratios becoming apparent. The phenomena may occur in further morphs not discussed in this paper (see Cain, this volume).

Clearly, in any future work, the progeny of a mating must be scored first regardless of the appearance of the parents, which may be misleading.

Lip colour morphs

In the course of the above breeding experiments, set up to investigate the genetics of the banding pattern, lip colour has also been scored, and the results are reported here.

Strictly, *T. pisana* does not form a well-marked lip as does *Cepaea,* but for the sake of simplicity, the thickening of the shell around the aperture will be described here as such. It may be either pink or within a range from almost white through buff to a pale brownish colour. Those with a pink lip are, in most cases, clearly separable from the others. Shells with only a small area of pink around the columella have been scored as pink, as have those with only very pale pink.

The results of all the matings are given in Table 2. Because some (or all) shells of the progeny of a mating were too small, fewer matings were scored for lip colour than for banding pattern, and the total scored for lip colour in a particular mating was in most cases less than for banding pattern. A number of matings of pink to pink gave only pink-lipped progeny (TP 154, 155, 160, TPR 213, 227), and of non-pink to non-pink gave only non-pink progeny (TP 172, 178, 183, TPR 217, 218, 221, F 5, 16, 19, 24, 26). Ratios of 1:1 were obtained in matings TP 152, 156 and 169, which were of pink to non-pink parents. TP 180 and F 11 probably belong in this group but only a low number of progeny could be scored. These matings simply indicate that both pink and non-pink breed true or segregate. The remaining four matings in which it was possible to score lip colour gave conflicting results. TP 171 (pink × non-pink) and F 29 (? pink × non-pink) gave all non-pink progeny, suggesting non-pink as the dominant allele; but TPR 216 and F 1 (both pink × pink) gave 3:1 ratios of pink to non-pink (with quite large numbers in F 1), suggesting pink as the dominant allele. If these 3:1 ratios are correct, the scoring of the parents of TP 171 and F 29 may have been

TABLE 2. Results of breeding experiments—lip colour.

Mating	Parental phenotypes	Progeny phenotypes	
		Pink	Non-pink
TP152	pink × non-pink	13	13
TP154	pink × pink	64	
TP155	pink × pink	1	
TP156	pink × non-pink	26	27
TP160	pink × pink	65	
TP169	pink × non-pink	27	32
TP171	pink × non-pink		48
TP172	non-pink × non-pink		16
TP178	non-pink × non-pink		18
TP180	pink × non-pink	2	9
TP183	non-pink × non-pink		25
TPR213	pink × pink	16	
TPR216	pink × pink	15	7
TPR217	non-pink × non-pink		5
TPR218	non-pink × non-pink		27
TPR221	non-pink × non-pink		12
TPR227	pink × pink	17	
F1	pink × pink	62	24
F5	non-pink × non-pink		17
F11	pink × non-pink	2	3
F16	non-pink × non-pink		34
F19	non-pink × non-pink		16
F24	non-pink × non-pink		9
F26	non-pink × non-pink		37
F29	?pink × non-pink		10

incorrect. However, if pink is dominant, both parents of both these matings must have been homozygous non-pink and expression of pink in the absence of an allele for pink seems unreasonable. If one parent in these matings was heterozygous pink/non-pink, a 1:1 ratio in the progeny would be expected, yet all the progeny are non-pink. These results cannot be explained further.

Pink and non-pink occur at similar frequencies in the banding classes segregating in individual matings (e.g. TP 156: plain unbanded—13 pink, 11 non-pink; dotty unbanded—5 pink, 4 non-pink; dark five-banded—8 pink, 12 non-pink). Pink/non-pink lip colour must therefore be at a further unlinked locus.

VARIATION IN THE FIELD AT TENBY (SOUTH WALES)

Methods

Samples taken from six sites at Tenby (Fig. 3) over five years (1977–1981) have been used to estimate frequencies at these sites of the morphs determined in the breeding experiments. Details of this sampling programme, with descriptions and precise locations of the sampling sites are given by Cowie (1982). Since *T. pisana* at Tenby is biennial and most individuals are semelparous, breeding in late summer and then dying, samples taken in late spring to mid-summer show a clear bimodal distribution of shell size; samples taken at other times of year have less distinct separation of the cohorts (Cowie, 1984). Samples used in the present study of morph frequencies were all taken between 24 May and 31 August of each year, and were all distinctly bimodal. Analysis of size distributions (Harding, 1949; Southwood, 1978) could not be performed on individual samples, since these were not large enough, but has allowed the trough in the distinctly bimodal size distributions of the combined samples on each sampling occasion to be estimated. (Ten samples were usually taken: two from site 1, three from site 2, one from site 3, two from site 4, and one each from sites 5 and 6.) Shells in each sample were allocated to the adult or juvenile age-group if they were larger or smaller respectively than the value at the lowest point of the trough in the distribution of the combined samples.

Adults were then scored as (i) plain unbanded, (ii) dotty unbanded, (iii) dark five-banded, (iv) intermediate five-banded, and (v) yellow five-banded. Allocation to one of the banded classes was by comparison with the yellow and dark bred material (above). Shells falling between the ranges exhibited within these clutches (yellow—TP 169; dark—TP 152, 156, 180, 183, TPR 216, 217, 220, F 28, 30) were scored as intermediate. Juveniles were simply scored as banded, since there is variation in the size at which the shell darkens; a small juvenile could be mis-scored as yellow, dark lineolations appearing somewhat later in its growth. All shells could be readily scored according to this scheme.

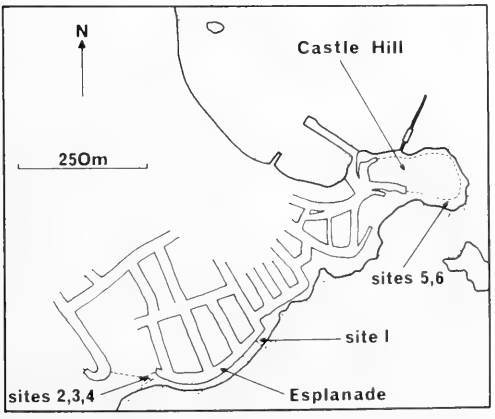

FIG. 3. Map of Tenby showing the positions of the sampling sites.

Results

The morph scores for each sample are available in Cowie (1982). Considering adults and juveniles separately, samples taken at a particular site during a particular year are not heterogeneous in morph frequencies (heterogeneity χ^2 tests on the actual numbers in each category—Cowie, 1982), and the data have therefore been pooled. Percentages of (i) unbanded, (ii) plain (within the unbanded class), (iii) dark (within the five-banded class), and (iv) yellow (within the five-banded class) are given in Table 3. The results are summarised in Figs. 4, 5 and 6. The adult and juvenile morph frequencies for each year at each site mostly fall within a fairly narrow range; extreme values are almost all from small samples.

There are no significant differences (heterogeneity χ^2 tests—Cowie, 1982) between

TABLE 3. Percentages of morphs, with 95% confidence limits. The 95% confidence limits have been calculated using the tables given by Sokal & Rohlf (1973), supplemented by Goldstein (1964). No samples were taken at sites 2 and 6 in 1980, and there were no adult unbandeds in the 1979 sample at site 6. Confidence limits have not been given where sample sizes were too small to allow their calculation.

		% unbanded		% plain		% dark	% yellow
Site	Year	ads.	juvs.	ads.	juvs.	ads.	ads.
1	1977	17.1 (13.7, 20.9)	14.5 (11.3, 18.2)	64.9 (53.0, 75.4)	73.8 (61.1, 84.1)	71.9 (67.0, 76.3)	5.6 (3.5, 8.4)
	1978	15.9 (11.4, 21.3)	17.9 (14.2, 22.2)	75.0 (58.8, 87.7)	65.6 (52.9, 76.9)	79.0 (72.5, 84.5)	2.1 (0.6, 5.3)
	1979	13.2 (5.5, 26.6)	32.6 (22.3, 44.4)	71.4 (39.0, 96.2)	50.0 (16.0, 84.0)	80.4 (67.8, 90.4)	4.4 (0.5, 14.4)
	1980	16.4 (8.6, 27.3)	15.4 (8.3, 25.2)	63.6 (32.9, 88.9)	50.0 (21.0, 79.0)	76.8 (63.7, 86.9)	8.9 (3.0, 19.6)
	1981	12.0 (4.5, 24.3)	22.6 (14.6, 32.4)	50.0	81.0 (58.4, 94.5)	70.5 (57.3, 82.6)	6.8 (1.4, 18.1)
2	1977	40.2 (36.4, 44.2)	43.6 (39.9, 47.5)	82.7 (77.7, 87.3)	85.1 (80.8, 89.6)	76.9 (72.3, 81.0)	5.1 (3.2, 7.7)
	1978	38.7 (34.3, 43.2)	40.6 (34.9, 46.5)	72.3 (65.2, 78.6)	80.4 (71.9, 87.2)	69.9 (64.4, 74.9)	7.2 (4.6, 10.7)
	1979	33.7 (24.5, 43.8)	32.6 (24.8, 41.1)	63.6 (46.0, 79.4)	74.4 (60.8, 86.1)	80.0 (68.4, 88.8)	6.2 (1.8, 14.9)
	1980	—	—	—	—	—	—
	1981	33.5 (28.8, 38.5)	41.7 (33.9, 49.9)	67.5 (58.5, 75.6)	74.6 (62.2, 84.6)	66.8 (60.5, 72.7)	8.4 (5.3, 12.6)
3	1977	29.5 (21.7, 38.4)	36.3 (28.0, 45.3)	66.7 (50.5, 81.3)	73.3 (60.4, 85.0)	72.1 (61.5, 81.1)	4.7 (1.3, 11.4)
	1978	32.4 (24.7, 40.8)	32.7 (25.8, 40.3)	86.4 (73.7, 94.8)	67.3 (53.4, 79.2)	76.1 (66.1, 84.4)	6.5 (2.5, 13.6)
	1979	66.7	30.2 (22.4, 38.9)	100.0	60.5 (45.8, 75.3)	50.0	0.0
	1980	30.4 (18.5, 42.8)	31.6 (22.5, 41.9)	64.3 (41.9, 86.6)	66.7 (47.2, 82.7)	71.9 (53.7, 86.2)	6.3 (0.8, 20.7)
	1981	26.9 (11.6, 47.5)	16.7 (0.4, 59.7)	71.4 (39.0, 96.2)	100.0	73.7 (51.4, 90.6)	5.3 (0.1, 25.3)
4	1977	34.4 (28.2, 41.1)	40.5 (33.2, 48.0)	68.9 (57.2, 79.0)	91.7 (82.8, 96.8)	73.8 (65.8, 80.7)	5.7 (2.5, 10.8)
	1978	33.0 (27.6, 38.8)	36.5 (32.2, 41.0)	61.1 (50.3, 71.0)	62.0 (54.4, 69.2)	78.1 (71.4, 83.8)	3.8 (1.6, 7.7)
	1979	30.9 (19.6, 44.7)	33.7 (27.1, 40.6)	70.6 (45.6, 89.5)	77.6 (66.0, 86.7)	79.0 (64.7, 90.3)	7.9 (1.7, 20.9)
	1980	46.4 (36.9, 56.0)	34.1 (27.1, 41.8)	66.7 (51.4, 79.3)	73.7 (60.4, 84.3)	74.6 (61.7, 84.9)	3.4 (0.5, 11.7)
	1981	29.0 (22.2, 36.5)	37.7 (26.6, 50.0)	55.3 (40.0, 70.0)	61.4 (41.0, 79.7)	72.2 (63.1, 80.0)	9.6 (4.9, 16.4)
5	1977	15.3 (10.6, 21.0)	22.1 (13.0, 33.6)	77.4 (59.1, 90.4)	86.7 (59.5, 98.3)	69.2 (61.8, 76.0)	8.7 (5.0, 13.9)
	1978	15.5 (9.7, 23.0)	17.8 (12.7, 25.0)	73.7 (51.4, 90.6)	58.8 (42.1, 75.0)	75.0 (65.6, 82.9)	8.7 (4.0, 16.0)
	1979	7.1 (0.2, 31.8)	45.5 (17.0, 73.9)	100.0	60.0 (14.7, 94.7)	71.4 (47.3, 91.3)	14.3 (1.8, 39.7)

TABLE 3 (Continued)

Site	Year	% unbanded ads.	% unbanded juvs.	% plain ads.	% plain juvs.	% dark ads.	% yellow ads.
	1980	13.2 5.5 24.8	21.4 4.3 46.6	57.1 11.0 81.0	33.3	76.1 63.4 87.0	6.5 1.4 17.3
	1981	20.0 6.8 40.7	50.0	80.0 28.4 99.5	50.0	75.0 56.3 94.3	15.0 3.2 37.9
6	1977	14.9 10.8 20.2	16.1 10.2 23.7	69.7 52.0 84.3	80.0 56.4 94.3	73.9 67.0 80.0	5.3 2.5 9.5
	1978	15.8 10.3 23.0	11.0 7.5 15.5	90.5 69.8 98.8	74.1 54.3 88.8	77.7 69.0 84.9	6.3 2.6 12.4
	1979	0.0 0.0 11.8	14.7 5.0 30.7	—	60.0 14.7 94.7	55.2 36.0 74.0	3.5 0.1 17.6
	1980	—	—	—	—	—	—
	1981	12.4 6.6 20.6	21.7 14.8 29.0	83.3 53.7 97.9	80.8 60.8 93.4	67.1 56.1 76.7	8.2 3.4 16.2

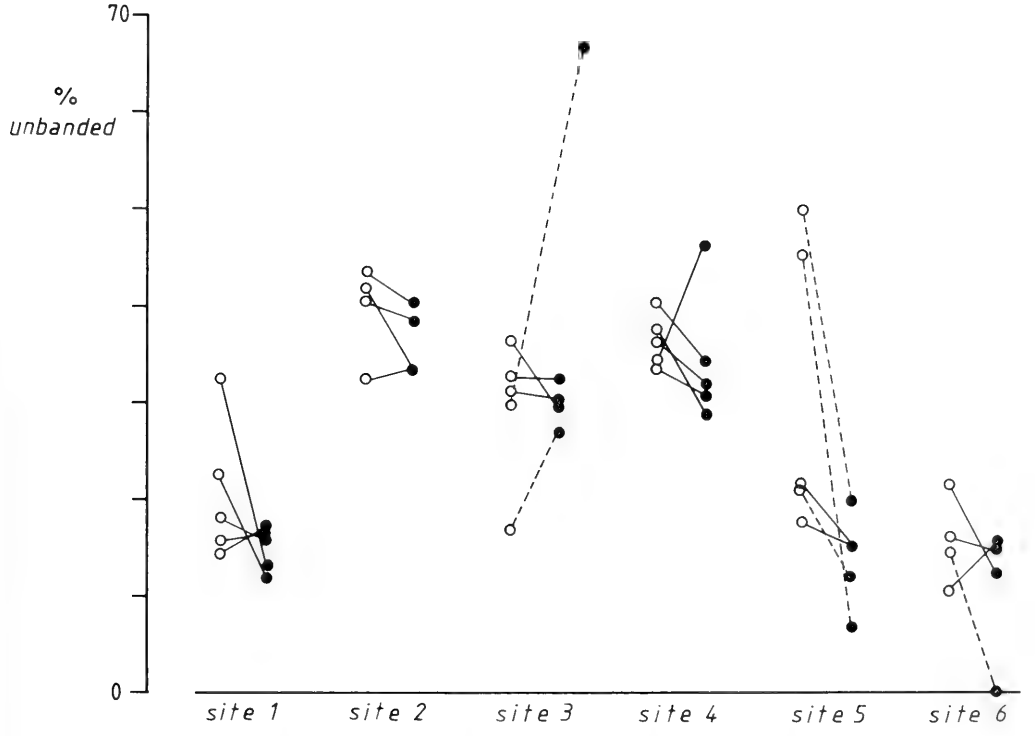

FIG. 4. Percentage unbanded, with values for adults (closed circles) and juveniles (open circles) connected in each year. Connections by broken lines are for years when the adult and/or juvenile value was based on a sample of less than 30.

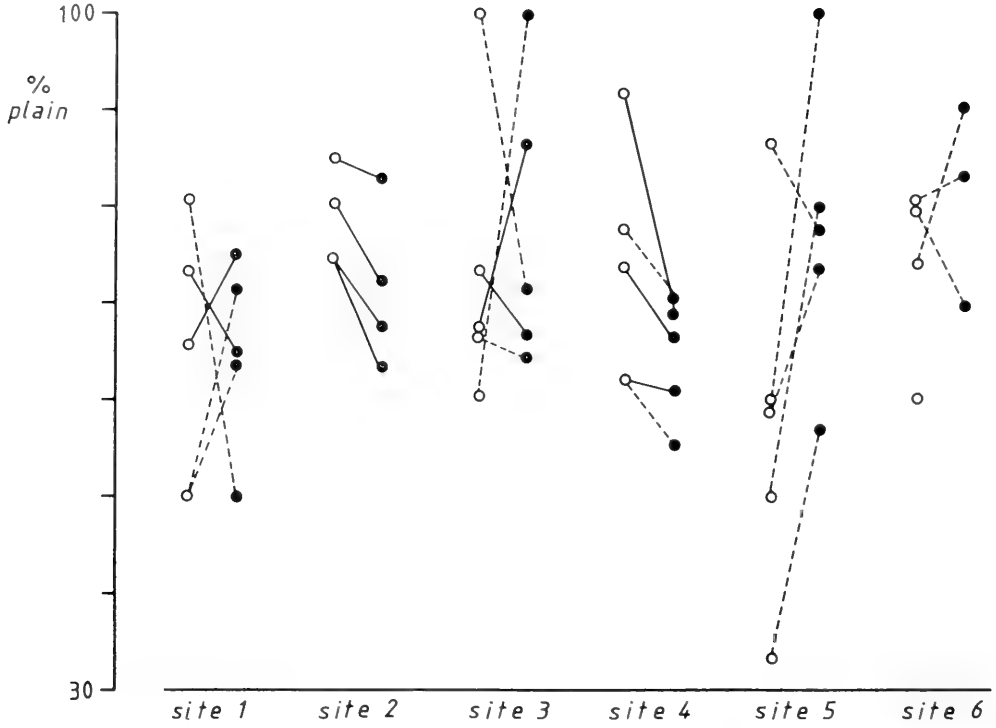

FIG. 5. Percentage plain within the unbanded class, symbolised as in Fig. 4. (The unconnected juvenile point for site 6 is for 1979 when no unbanded adults were sampled at this site.)

sites within any particular year in the frequencies of plain and dotty within the unbanded shells (either adults or juveniles), nor of dark, intermediate and yellow within the five-banded class (only adults scored). However, there is significant heterogeneity between sites in the frequencies of unbanded and five-banded shells in both adults and juveniles ($p << 0.05$ on most occasions). The sites appear to fall (Fig. 4) into two groups: 1, 5 and 6, and 2, 3 and 4. Comparing between sites and within years, adult and juvenile frequencies of all morphs are homogeneous (but see next paragraph). At some sites, there is heterogeneity between years in some morph frequencies (site 1, juveniles, unbanded/banded, $p < 0.001$; site 2, adults, plain/dotty, $p < 0.005$; site 2, adults, dark/intermediate/yellow, $p < 0.025$; site 4, juveniles, plain/dotty, $p < 0.001$). This is more than would be expected by chance, as shown by Cooper (1968) in his discussion of significance levels in multiple χ^2 tests made simultaneously. However, there is no consistent pattern in these differences, and indeed the 95% confidence limits of the percentages for all the years at any particular site almost all overlap. There is no detectable trend in changes of morph frequencies at any site in either adults or juveniles; they remain similar over the five years. Sign tests on the differences between the 1977 and 1981 percentages for each morph showed no significant trend. The heterogeneity is not understood.

Although comparison within sites and within years indicates no heterogeneity between adults and juveniles in the frequency of unbanded shells, there is a slight suggestion from Fig. 4 that it may be higher in the juveniles. A Wilcoxon's signed-ranks test on the adult/juvenile differences over all sites and all years showed a higher percentage of unbandeds in the juveniles than in the adults ($p < 0.02$); this is not due to mis-scoring of either adults or juveniles, since the unbanded/banded distinction is very clear. However, *T. pisana* at Tenby is essentially biennial and

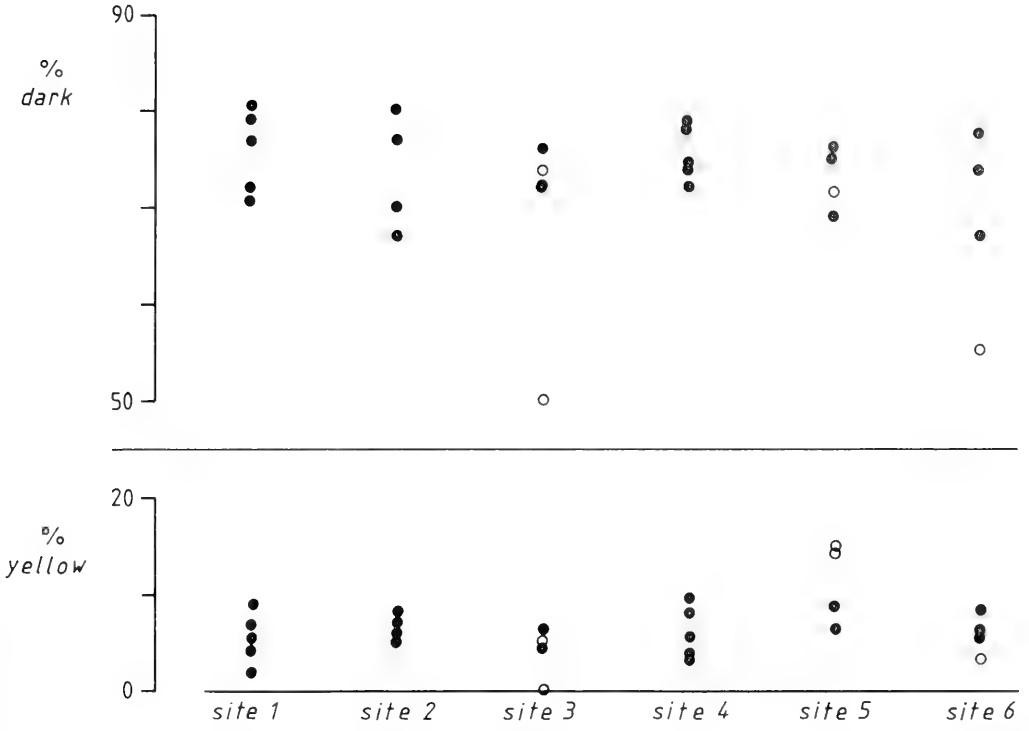

FIG. 6. Percentages of dark and yellow within the banded class (adults only scored). Closed circles represent percentages based on samples of over 30 individuals, open circles those on under 30.

semelparous, and although some snails may mature in only one year or require three to reach maturity, thus connecting the two gene pools, it is possible that one-year-old and two-year-old snails may belong to somewhat different populations genetically. Therefore, the percentage unbanded in the adults of each year was also compared with that in the juveniles of the previous year (i.e. the population from which the adults were derived) at each site. Again, a Wilcoxon's signed-ranks test showed a significant decrease in the proportion of unbandeds from one-year-old to two-year-old snails ($p < 0.02$). The 20 possible comparisons are illustrated graphically in Fig. 7. There is a clear tendency for the points to fall above the line of equal pecentage unbanded.

Similar comparisons were carried out for plain and dotty within the unbandeds. The superficial tendency for the proportion of plain to decrease from one-year-old to two-year-old snails, suggested by Fig. 5, was not quite significant at the 5% level.

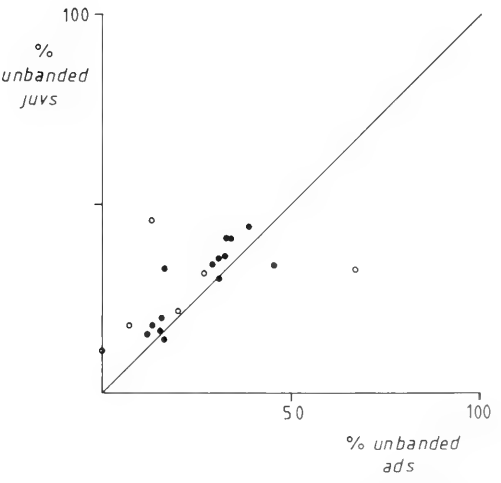

FIG. 7. Scatter diagram of percentage unbanded in the adults in each year against that in the juveniles of the previous year at each site, with the line of equal percentages drawn in. Open circles—adult or juvenile sample <30.

Discussion of variation at the main Tenby sites

The small but significant indication overall of a greater proportion of unbanded shells in the one-year-old (juvenile) class than in the two-year-old (adult) class, suggests selection in the second year, at least, of life. (It has not been possible to obtain sufficiently reliable scores for very young shells to allow selection, if acting, to be detected in the first year of life.) Genetic drift, although possible on the basis of a minimum estimate of neighbourhood size (*sensu* Wright, 1969) in the Tenby colony (minimum estimate of N_e = 115 individuals—Cowie, unpublished), would not produce this overall trend, since changes in gene frequencies at the different sites would, by definition, be random. Selection in favour of banded shells would be expected if, at the northern edge of the species' range, animals capable of absorbing more solar radiation (i.e. those with banded shells) were at an advantage (Kettlewell, 1973; Jones, 1973a,b; Heller & Volokita, 1981a,b). This advantage could work in various ways; one might be that banded snails grow to a larger size than unbandeds, but no indication of this has been found (Cowie, 1982).

It is also possible that selection is by visual predation, banded shells being more cryptic (at least to the human eye—Heller, 1981). Certainly, both avian and mammalian predators are present in the vicinity. If visual predation is a factor, it could act in a density- or frequency-dependent manner (e.g. Clarke, 1972, 1975) as discussed for *Cepaea* by Jones, Leith & Rawlings (1977) and Clarke, Arthur, Horsley & Parkin (1978). No correlation has been found between the degree of selection (as indicated by the difference in proportion of unbandeds in adults and juveniles), and either the adult or juvenile population densities at each site. However, the estimates of density (Cowie, 1982) are very variable (adults—39 to 202 m^{-2}, juveniles—13 to 436 m^{-2}), and the above data on the degree of selection are few; a definite conclusion about density-dependence is almost certainly not justified. There is no indication of frequency-dependent selection, since Fig. 7 does not show any tendency for points to be at different distances from the equal percentage line, or on opposite sides of it, according to morph frequencies (cf. Cook, 1965); again, the data may be too few, although at the high densities at which *T. pisana* exists at Tenby, apostasy may be of little importance (Greenwood, 1969).

The possible selection favouring dotty over plain unbandeds is in the expected direction if snails with more banding are at an advantage.

Since the Tenby colony may have been introduced within only the last few hundred years (Cowie, 1982), it is possible that gene-frequency equilibrium has not been reached, and the proportion of banded snails is gradually increasing because of selection, but that this trend was not detected in the five years of this study. Alternatively, gene frequencies may be stable, and the selective advantage during the second year of life of the banded snails is balanced, at whatever is the appropriate frequency for the particular site, by selection favouring unbandeds during their first year, or by greater productivity of unbanded snails, although the latter is contrary to expectation if banded snails can absorb more energy from the sun (cf. Heller & Volokita, 1981a: 274).

The proportions of unbanded shells within both adults and juveniles are clearly different at sites 1, 5 and 6, and sites 2, 3 and 4. Sites 2, 3 and 4 are close together at the western end of the Esplanade, and may all be located within a single population. Sites 5 and 6 are close to each other on Castle Hill and could certainly be within a single population. The population at site 1 (towards the eastern end of the Esplanade) must be distinct from the others, despite its similarity in morph frequency with sites 5 and 6. It is likely, therefore, that the six sites are located within at least three breeding populations: at site 1, at sites 2, 3 and 4, and at sites 5 and 6. There are no obvious differences between these three groups of sites, although no special study has been made of habitat characteristics. Site 3 is the least exposed and most shaded, yet its morph frequencies are similar to those of sites 2 and 4, which are as exposed as the others. These similarities and differences between sites may be due to founder effects, population bottlenecks, or to selection. However, the overall selection in favour of banded shells does appear to be superimposed upon these between site differences.

VARIATION AT OTHER BRITISH LOCALITIES

Samples have been taken, or records made in the field, at a number of other sites

both in and around Tenby, and elsewhere in the British distribution of *T. pisana* (Kerney, 1976; Cowie, 1982). At all but two localities (dealt with individually below), shells could be readily scored according to the scheme of morphs developed for the main six Tenby sites. (The scores are given in Cowie, 1982).

Within Tenby (detailed distribution given by Stubbs, 1900; re-surveyed by Cowie, 1982), both banded and unbanded shells were usually present (five sites, some sampled more than once), but sometimes in different proportions than at the six main sites. In the unbanded class, frequencies of plain and dotty shells were similar to those at the six main sites, as were frequencies of dark, intermediate and yellow shells within the banded class. In South Wales, but outside Tenby (Caldey Island, Saundersfoot, Stackpole Warren, Porthcawl-Newton), only banded shells were found, these mostly dark, except at Saundersfoot where they were yellow and intermediate only (cf. Dean, 1916).

At all these localities, shell colour is pale yellowish-buff to off-white, and banded shells always have darker apices than unbandeds. Apex colour is probably very closely linked to the unbanded/banded locus, and not a pleiotropic effect of it, since shells from other localities may have the opposite combination of unbanded/banded and apex colour, and apex colour segregates in bred material in bandeds and unbandeds (see below, and Cain, this volume). If sufficient time has elapsed since the founding of these colonies for crossing-over to have taken place, this association implies selection maintaining the combination of dark apex and banded and pale apex and unbanded at these localities. This may reflect the underlying linkage disequilibrium between the loci controlling this character.

At Porthcawl-Kenfig (Glamorgan) and St. Ives (Cornwall) the shells did not fit the Tenby scoring scheme. The sample from Kenfig contains both five-banded and unbanded shells, but there is no clear distinction between them as at Tenby. At Tenby, banded shells are banded right from the embryonic shell, even if without dark lineolation; their apices are dark, and the shell colour is pale yellowish-brown. At Kenfig, there is a continuum from shells with bands right from the embryonic shell, via those with bands starting later, to those with no bands at all; most shells have a pale apex, and the shell colour is much more white. The shells appear more similar to those from the Baie du Mont St. Michel (Normandy) than to any British ones (see below). The elucidation of the control of this variation must await breeding experiments. This is the only British locality outside Tenby at which unbanded shells have been found.

At St. Ives, only two forms, both five-banded, are present: one with full pigmentation, and one described as subhyalozonate by Cain (this volume). They occur in roughly equal proportions in the population. The banding pattern of both forms appears the same, the subhyalozonate almost, but not entirely, lacking pigment in both bands and shell background. It appears white, and effectively unbanded, usually with a very pale apex, although with the animal inside the shell it is considerably darkened. The normally-pigmented form falls within the intermediate class in darkness of bands when compared with Tenby material, but is more similar to shells from Brittany than to those from Tenby in pattern (see below). Normal pigmentation is dominant to subhyalozonate (Cain, this volume). Fig. 8 shows banded shells from Tenby, Kenfig and St. Ives for comparison.

VARIATION AT NON-BRITISH LOCALITIES

Northern France and the Channel Islands

Detailed locations of all sites in Brittany, Normandy and the Channel Islands are given in Cowie (1982).

The Breton samples (five from the Baie du Quiberon area, one from Roscoff) consist largely of banded shells, two of them wholly so. Not unexpectedly, the proportions of unbanded and banded shells appear different at the different sites. All the banded shells are five-banded. There is some variation between sites in the overall appearance of these banded shells, but although all are similar to some of the intermediate banded shells from Tenby, none is quite the same. The band lineolations are less continuous than at Tenby, and the shells have a more speckled appearance. In fact the normally-pigmented shells from St. Ives resemble these extremely closely.

Further forms, not found in Britain, are present at low frequency in some of these samples from Brittany (cf. Sacchi & Gaudiosi,

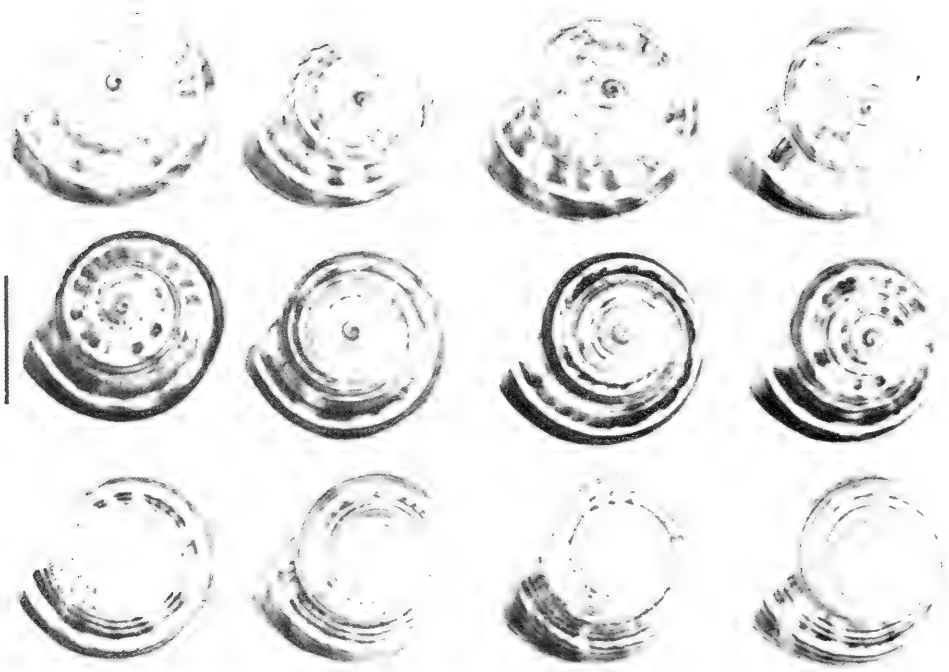

FIG. 8. Banded shells from St. Ives (top), Tenby (middle) and Kenfig (bottom).

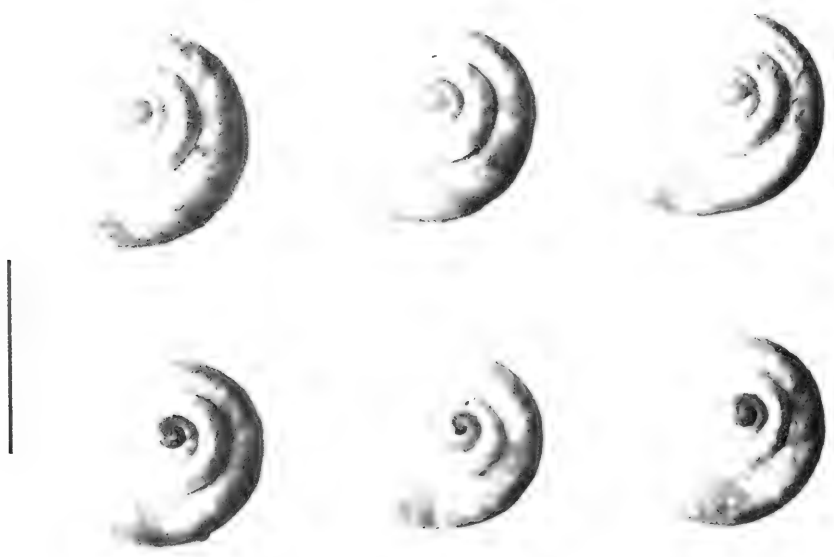

FIG. 9. Shells from the progeny of mating TPR 202 with pale apices (upper row) and dark apices (lower row), the latter showing the range of variation in the amount of dark pigment.

1961). A true hyalozonate, as well as subhyalozonate, appears as both banded and unbanded, suggesting its control at a different locus. As unbanded it is extremely white, and the apex colourless. The distinction between hyalozonate and subhyalozonate is clear in the bandeds but less so in the unbandeds.

A single sample from Guernsey (Port Soit) consisting of normally-pigmented five-banded shells only, shows, not unexpectedly, great similarity to the style of banding of the Breton samples. (*T. pisana* was originally introduced to Guernsey from Jersey in 1860—Barrett, 1972.)

The single sample from Normandy (Baie du Mont St. Michel) is different from those from Brittany, showing strong similarities to that from Porthcawl-Kenfig, in that it is difficult to draw a line between unbanded shells, shells with bands on only later whorls, and five-banded shells, and the background shell colour is whiter. Shells with dark and pale apices are present within both the unbanded and banded classes.

In some shells in all these northern French samples there is a slight tendency for band 3 to be rather darker and less broken up than bands 1 and 2. This character is not seen in Britain, except perhaps at Porthcawl-Kenfig.

The major region of the distribution of *T. pisana*

Forms similar in banding pattern to those found in northern Europe are known from this area, which includes the Mediterranean, and the Atlantic coasts of North Africa and the southern Iberian peninsula. However, many other forms are also known, although most samples contain only some of them. A good selection of the immense range has been illustrated by Taylor (1912), although his pictures show little fine detail. Forms with band 3 heavily emphasized and with 1 and 2 very much reduced or absent, are common, as are forms with bands reduced to flecks, these flecks sometimes apparently superimposed on a yellowish background band; a common version of this type has heavy flecks on the upper surface of the shell on bands 1 and 2 and not on band 3. Forms with only bands 4 and 5, or 5 only, are well-known, and some shells appear truly unbanded, even lacking the very late bands often found towards the shell aperture of unbanded shells from Tenby. (For further details of some of these forms, and their genetic control, see Cain, this volume.)

The other major difference between shells from this region and those from the north, is that the background shell colour can often be very white, although buff and off-white colours, as in the British and French samples, are sometimes found. Tan and pink varieties are also known.

Apex colour varies from nearly black to almost colourless (colourless in hyalozonate forms), and different apex colours appear to segregate clearly in some samples (Table 4) in both banded and unbanded shells, suggesting that they are not pleiotropic effects of the unbanded/banded locus, as material from Tenby could imply (above). The implied linkage disequilibria between the banding and apex colour loci in some samples (Table 4) suggests selection favouring particular combinations, the predominant associations being of banded with dark apex, and unbanded with pale apex. A single cross (TPR202) of two unbanded shells with pale apices, from Lesbos (Greek Islands), gave a 3:1 ratio of pale:dark (62 pale:25 dark) in the progeny (all unbanded); dark, which can vary consider-

TABLE 4. Examples of apex colour scores in unbanded and banded shells (diameter > 12 mm) from various localities.

	Unbanded		Banded	
Sample	Dark apex	Pale apex	Dark apex	Pale apex
Lesbos	21	50	26	0
Amnissos 2 (Crete)	0	48	8	0
Elviria 1978 (S. Spain)	7	48	2	4
Elviria 1979	4	39	3	4

ably in the intensity and amount of pigmentation, is thus recessive to pale (Fig. 9).

CONCLUDING DISCUSSION

The variation of *T. pisana* makes sorting of large samples into distinct forms extremely difficult without knowledge of the genetic control. We are only beginning to understand this control; it may be much more complex than that of the more limited variation in *Cepaea*. This study has broadly characterised genetically the relatively simple variation at Tenby, and Cain (this volume) has investigated the genetics of some forms from the Mediterranean, northern France and St. Ives. Few crosses have been made between animals from these different regions, and so, although some forms are not present at certain localities, it is impossible to be sure whether slightly different forms are indeed different morphs, or simply the same morph expressed against rather different genetic backgrounds and/or influenced by environmental variation. For instance, the five-banded shells from Britain and northern France, and some from the Mediterranean, although having distinct regional characters in the styling of the banding pattern, may not be distinct morphs, and may even be genetically identical. Similarly, unbanded shells from Tenby with some banding appearing late on the body whorl in many cases, may be the same morph as completely unbanded shells from elsewhere, but expressed against a different background. On the other hand, there may be different morphs, in which case the nomenclature may have to be modified, and the Tenby morph designated as something other than unbanded. The genetic control of this kind of minor variation may be complicated.

Since variation in natural selection may be even more localised in *T. pisana* than in *Cepaea* (Heller, 1981), and differential habitat selection by different morphs may enhance the large differences in morph frequency over short distances (Johnson, 1980, 1981), it is not possible to use single samples to give representative morph ratios for a whole region. However, it is still possible to detect differences and similarities between localities. The clear similarities between shells from St. Ives, Guernsey and Brittany, and between shells from Porthcawl-Kenfig and Normandy, support the suggestion from other evidence that *T. pisana* has been introduced only recently to these British sites (Cooke, 1916; Turk, 1972; Cowie, 1982), although the exact provenance of these British colonies cannot be established. All other British shells bear strong resemblance to those from Tenby, and may be the result of introductions from there. Tenby shells, although similar to those from northern France, bear no strong resemblance to either these or to Mediterranean shells. It is possible that *T. pisana,* even if introduced to Tenby as well as to the other British localities, may have been there long enough for selection to have given rise to a range of variation characteristic of this locality.

In general, these northern European populations exhibit less variation than those in the Mediterranean. Assuming that *T. pisana* originated in the Mediterranean region, perhaps in Morocco (Sacchi, 1971), the reduced number of forms seen in the northern populations (probably artificially introduced), must result from founder effects and/or natural selection of the few forms capable of withstanding the more temperate condition (cf. introduced colonies of *Cepaea nemoralis* in North America—Brussard, 1975). Some Mediterranean localities also have only a small number of forms, probably for similar reasons if the species spread from a small area of origin in the western Mediterranean.

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REFERENCES CITED

BARRETT, B. W., 1972, *Theba pisana* in Guernsey, 1971. *Journal of Conchology,* 27:391–396.

BRUSSARD, P. F., 1975, Geographic variation in North American colonies of *Cepaea nemoralis. Evolution,* 29: 402–410.

CAIN, A. J., 1981, The development of systematic ideas of variation illustrated by malacology. In: WHEELER, A. & PRICE, J. H. eds., *History in the service of systematics.* Society for the Bibliography of Natural History, London, p. 151–156.

CAIN, A. J., 1984, Genetics of some morphs in the land snail *Theba pisana. Malacologia,* 25: 381–411.

CAIN, A. J., KING, J. M. B. & SHEPPARD, P. M., 1960, New data on the genetics of polymorphism in the snail *Cepaea nemoralis* L. *Genetics,* 45: 393–411.

CLARKE, B., 1972, Density-dependent selection. *American Naturalist,* 106: 1–13.

CLARKE B., 1975, Frequency-dependent and density-dependent natural selection. In: SALZANO, F. M. ed., *The role of natural selection in human evolution.* North-Holland Publishing Co., p. 187–200.

CLARKE, B., ARTHUR, W., HORSLEY, D. T. & PARKIN, D. T., 1978, Genetic variation and natural selection in pulmonate molluscs. In: FRETTER, V. & PEAKE, J. eds., *Pulmonates,* 2A. Academic Press, London, New York, San Francisco, p. 219–270.

COOK, L. M., 1965, A note on apostasy. *Heredity,* 20: 631–636.

COOKE, A. H., 1916, A new British locality for *Helix (Euparypha) pisana* Müll. *Proceedings of the Malacological Society of London,* 12: 4.

COOPER, D. W., 1968, The significance level in multiple tests made simultaneously. *Heredity,* 23: 614–617.

COWIE, R. H., 1980, Precocious breeding of *Theba pisana* (Müller) (Pulmonata: Helicidae). *Journal of Conchology,* 30: 238.

COWIE, R. H., 1982, *Studies on the ecology and ecogenetics of the helicid land snail* Theba pisana *(Müller).* Ph.D. thesis, University of Liverpool.

COWIE, R. H., 1984, The life-cycle and productivity of the land snail *Theba pisana. Journal of Animal Ecology,* 53: 311–325.

DEAN, J. D., 1916, Notes on *Helix pisana* Müller and its occurrence at Porthcawl. *Journal of Conchology,* 15: 85–86.

DEBLOCK, R. & HOESTLANDT, H., 1967, Données biologiques sur le gastropode littoral *Theba pisana* Müller aux limites septentrionales de son extension. *Comptes rendus de l'Académie des Sciences,* Paris, 265: 893–896.

GERMAIN, L., 1929, Les Helicidae de la faune française. *Archives du Muséum d'Histoire Naturelle de Lyon,* 13: 1–484.

GOLDSTEIN, A., 1964, *Biostatistics,* Macmillan, New York, ix + 272 p.

GREENWOOD, J. J. D., 1969, Apostatic selection and population density. *Heredity,* 24: 157–161.

HARDING, J. P., 1949, The use of probability paper for the graphical analysis of polymodal frequency distributions. *Journal of the Marine Biological Association of the United Kingdom,* 28: 141–153.

HELLER, J., 1981, Visual versus climatic selection of shell banding in the landsnail *Theba pisana* in Israel. *Journal of Zoology,* 194: 85–101.

HELLER, J., 1982, Natural history of *Theba pisana* in Israel (Pulmonata: Helicidae). *Journal of Zoology,* 196: 475–487.

HELLER, J. & VOLOKITA, M., 1981a, Gene-regulation of shell banding in a land snail from Israel. *Biological Journal of the Linnean Society,* 16: 261–277.

HELLER, J. & VOLOKITA, M., 1981b, Shell-banding polymorphism of the land snail *Xeropicta vestalis* along the coastal plain of Israel. *Biological Journal of the Linnean Society,* 16: 279–284.

JOHNSON, M. S., 1980, Association of shell banding and habitat in a colony of the land snail *Theba pisana. Heredity,* 45: 7–14.

JOHNSON, M. S., 1981, Effects of migration and habitat choice on shell banding frequencies in *Theba pisana* at a habitat boundary. *Heredity,* 47: 121–133.

JONES, J. S., 1973a, Ecological genetics of a population of the snail *Cepaea nemoralis* at the northern limit of its range. *Heredity,* 31: 201–211.

JONES, J. S., 1973b, The genetic structure of a southern peripheral population of the snail *Cepaea nemoralis. Proceedings of the Royal Society of London,* B, 183: 371–384.

JONES, J. S., LEITH, B. H. & RAWLINGS, P., 1977, Polymorphism in *Cepaea:* a problem with too many solutions? *Annual Review of Ecology and Systematics,* 8: 109–143.

KERNEY, M. P., 1976, *Atlas of the non-marine Mollusca of the British Isles.* Institute of Terrestrial Ecology, Cambridge. v + (3) + 200 p., maps.

KETTLEWELL, B., 1973, *The evolution of melanism.* Clarendon, Oxford, xxiv + 423 p., 72 pl.

MURRAY, J., 1975, The genetics of the Mollusca. In: KING, R. C. ed., *Handbook of Genetics,* 3. Plenum, New York, p. 3–31.

SACCHI, C. F., 1971, Écologie comparée des gastéropodes pulmonés des dunes Méditerranéennes et Atlantiques. *Natura, Milano,* 62: 277–358.

SACCHI, C. F., 1977, La 'lacune nord-Adriatique' et son influence sur l'écologie des gastéropodes dunicoles. Prémisses methodologiques. *Atti della Società Italiana di Scienze Naturali, e del Museo Civile di Storia Naturale, Milano,* 118: 213–225.

SACCHI, C. F., 1978, Il delta del Po come elemento disgiuntore nell'ecologia delle spiaggie adriatiche. *Supplemento al Bollettino del Museo Civico di Storia Naturale di Venezia,* 29: 43–73.

SACCHI, C. F. & GAUDIOSI, M. R., 1961, Sul significo ecologico dell'azoto conchigliare nella elicide dunicola *Euparypha pisana* (Müll.)—II. Analisi di fenotipi differenti in una singola popolazione. *Rendiconti della Classe di Scienze fisiche, matematiche e naturali, Accademia Nazionale dei Lincei,* ser. 8, 30: 571–575.

SACCHI, C. F. & VIOLANI, C., 1977, Ricerche ecologiche sulle elicidi dunicole della Ria di Vigo

(Spagna) (Gastropoda Pulmonata). *Natura, Milano,* 68: 253–284.
SOKAL, R. R. & ROHLF, F. J., 1973, *Introduction to biostatistics.* Freeman, San Francisco, xiii + 368 p.
SOUTHWOOD, T. R. E., 1978, *Ecological methods.* Ed. 2. Chapman & Hall, London, xxiv + 524 p.
STUBBS, A. G., 1900, The land and freshwater shells of Tenby, South Wales. *Journal of Conchology,* 9: 321–328, 358–365.
TAYLOR, J. W., 1912, *Monograph of the land and freshwater Mollusca of the British Isles.* 3, part 19. Taylor Bros., Leeds, p. 369–416, 4 pl.
TURK, S. M., 1972, A study of the white sand-hill snail, *Theba pisana. Devon Trust for Nature Conservation Journal,* 4: 138–142.
WOLDA, H., 1969, Genetics of polymorphism in the land snail, *Cepaea nemoralis. Genetica,* 40: 475–502.
WRIGHT, S., 1969, *Evolution and genetics of populations. II. The theory of gene frequencies.* Chicago University Press, Chicago & London, vii + 511 p.

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GENETICS OF SOME MORPHS IN THE LAND SNAIL *THEBA PISANA*

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ABSTRACT

The exceptionally variable snail *Theba pisana* is abundant in Mediterranean and west European juxtalittoral habitats, and has been introduced elsewhere. So far, its banding variation has defied description and analysis; basically it has five bands as in other helicids. A breeding programme over several years has allowed a number of morphs to be characterized, including unbanded, forms with band 3 enhanced, others with it reduced, some with an additional line of dots at the lowest level of band 3, and fully five-banded. A hyalozonate and subhyalozonate form are also described. There can be considerable variation in the expression of most morphs, caused at least in part by genetic modifiers, so that, unlike in *Cepaea,* random samples often show apparently continuous variation. This explains the difficulty previous workers have had in scoring morphs; its significance for the animal is discussed.

INTRODUCTION

Although many species of land-snail show considerable variation in the colour and patterning of the shell, of interest to evolutionists, few have been investigated genetically (see Murray, 1975, for a review). Many species are difficult to rear, regular attention to the broods is required, and the generation time is very long when compared with that of *Drosophila* or bacteria. For some years, the extremely variable snail *Theba pisana* (Müller) has been bred in my laboratory with some success, to investigate the nature of its variation, which differs considerably from that of the well-known *Cepaea nemoralis* (L.). The species occurs almost entirely in juxtalittoral habitats (see Cowie, 1982 and Cowie, this volume). The background on which it is found is therefore less varied than those of *C. nemoralis* (woods, hedges, rough herbage, scree, open grassland) and in most colonies the shell colour ranges only from white through cream and buff, sometimes to brown. The banding, however, although of the same general plan (five bands with well-marked characteristics of breadth and position, see Cowie, this volume, fig. 1), has a different appearance and vastly greater variation. Each band except the very narrow band 1 is normally broken up into two or more separate lines (see e.g., Sacchi, 1952, 1955 for a schematization of these bandlets, and Taylor, 1912, for coloured plates) and these in turn may be feathered, or broken into dashes or dots which may fuse to chevrons and arrowheads. Moreover, these markings, usually darkly pigmented, are laid upon brownish yellow bands (see Cowie, this volume, fig. 2), which may show between the darker markings or be present without them; in breadth and position these yellow bands correspond to the solid dark bands of *Cepaea* but are not known in that genus, nor, as far as my experience goes, in other helicine snails.

Moreover, whereas it is usually easy to sort random samples of *Cepaea* by shell colour and banding types, it is seldom possible to do so with *T. pisana.* Even in the populations at Tenby reported by Cowie (this volume) some distinctions are difficult. Apparently clear-cut forms such as unbanded (00000) may be connected, by individuals having a trace of band 5 (the lowermost) only on the last whorl, to others with a well developed 5, or 5 and 4, and to yet others with all the bands (formula 12345) appearing in the last whorl. What, then, is the nature of the variation? It could be phenotypic, or polygenically controlled, or with major genes as in *Cepaea nemoralis* and *C. hortensis* but blurred by background genetic variation, or any combination of these agents. This paper describes the results of some years of breeding work on this species, undertaken to find answers to this question. Except for a few matings from St. Ives, Cornwall, all the material reported came from France, Spain and the Balearic Islands, and in this respect is complementary to that reported by Cowie (this volume).

MATERIALS AND METHODS

Collections of live snails made at various stations in the Mediterranean region and elsewhere were examined for possible distinctive forms, which were set up as breeding pairs if juvenile, or, if adult, as stock from which to obtain juveniles. It was not realized at first that, unlike any other helicid snail which has been bred, *T. pisana* can mate successfully while still apparently juvenile (Cowie, 1980). There is no record of self-fertilization in *T. pisana,* and much evidence against it (Cowie, 1982). Unless otherwise stated, all the matings reported here are of juveniles of less than 10 mm maximum shell diameter and growing well, individuals below this size being found by dissection to carry no sperm in the common duct (Cowie, 1982).

This means that selection of individuals for breeding has to be done while they are small. As they grow, the bands beneath the periphery, 4 and 5, are progressively concealed by the new whorls of the shell, and coated over by shell material, being visible only on the latest whorl of the shell. For this reason, only the upper side of the shell is usually scored in this paper, the bands above the periphery being completely visible. In fact, forms in my breeding material are often more distinguishable when the shell is small, the addition of bands on the body-whorl (or earlier) frequently tending to make them more alike. As the animals are often very prolific, many broods were killed at a modal shell diameter of about 6–8 mm or less, it being impossible to separate out hundreds of babies in order to grow them to maturity at suitably low densities per box.

Since some forms (e.g. hyalozonate) can be scored even on the embryonic shell, while others require at least one whorl or even two additional to it before they can be scored reliably, there is not infrequently an apparent discrepancy between totals for different forms as given in the various tables. Within such forms as 123, the size for reliable scoring may vary from brood to brood, depending on how early the bands are expressed. In a very few cases the uppermost whorls were broken in the extraction of the animals and some characters, e.g. apex colour, or slight dotty, could not be scored on these individuals.

The breeding data are given in Tables 1–10, in some detail because these molluscs take too long to breed for the work to be easily repeated.

SYMBOLS AND NOMENCLATURE

Reasons for describing the shell as five-banded (pentataeniate, as in other helicids) *pace* Sacchi (1952) and Heller (1981) are given by Cowie (this volume). The complexity of the banding variation requires an extension to the standard nomenclature used in *Cepaea* (e.g. Cain, Sheppard & King, 1968, and references therein). The standard numerical formula used for all pentataeniate shells, numbering from above downwards, is retained, an actual number showing the presence of a darkly pigmented band, and 0 being substituted when the band is absent. Additionally, in *T. pisana,* y is used when the band is present in brownish-yellow but has no dark band-markings on it. Thus 00y45 is a shell with 1 and 2 absent, 3 represented only by a yellow band, and 4 and 5 present with some black markings. These last two may have the markings superimposed on a yellow band, but if the markings are extensive, the yellow may be invisible, and it is not, therefore, normally scored under them. For brevity, the dark markings, whatever they may be, corresponding to a particular band are simply called a dark band. It has been customary for a long time in *Cepaea* to represent a band which is interrupted or incomplete by a colon. This is not good practice since it covers at least two very different conditions, (i) that in which the band is so weakly developed that part of it is absent, though what is present is usually continuous, and (ii) a punctate band, broken up into regular blocks or dots, which needs its own symbol. The first seems to be polygenically controlled, the second is an allele at a major locus, *I* (Cain, Sheppard & King, 1968). In *T. pisana,* the bands, instead of being solid as is usual in *Cepaea,* are normally broken up into parallel bandlets, a condition not found at all in *Cepaea,* and these are themselves frequently regularly or irregularly discontinuous. To represent almost all the bands in *T. pisana* by colons seems unnecessary, provided it is remembered that this discontinuity is their normal state. In the material discussed, only band 3 in the form 003 is normally solid (e.g. Fig. 8). (As mentioned above, in the work reported here the two last bands are often disregarded; a three-number formula refers to bands 1, 2 and 3.) What is useful is to indicate bands which are markedly more interrupted than in some standard. In this paper, the common form with all bands well represented and darkly pigmented (Cowie's dark 5-

TABLE 1. Results of matings. Key to mating letters and symbols: H, juveniles collected by John Humphries, St. Ives, Cornwall, England. Majorca, juveniles collected by Dr. M. A. Carter, Ses Coretes, south-east Majorca, Balearic Islands, Spain. MC, descendants of samples collected by Dr. M. A. Carter, St. Benoit des Ondes, Baie du Mont St. Michel, Normandy, France. PB, originals (with compound number, e.g. PB 1 2) and descendants of sample collected by Dr. P. Brakefield, Santa Cristina de Aro, c. 8 km. inland from Playa d'Aro, Gerona Province, Spain. EP 27, descendants of samples collected by A. J. C. near Montpellier, France. TP 8, mating between PB and EP 27 stock. TP 47, descendants of sample collected by Dr. S. Meredith, Luz, Portugal. All other TP matings shown are descended as indicated from these stocks.

Mating no.	Parents			Progeny		
	Source	Score	Size	Total	Score	Numbers
PB 12	PB 1/2	plain 000	11.1	294	plain 000	208
	PB 1/2	plain 000	13.4		dotty 000	1
					plain ::3	83
					dotty ::3	2
PB 26	PB 2/2	plain 000	11.9	37	plain 000	19
	PB 2/2	plain ::y	9.8		plain ::y	18
TP 3A	EP 27	plain 003	9.6	59	plain 003	51
	PB 12	plain ::3	5.7		dotty 003	8
TP 3B	TP 3A	? 003	?	92	plain 003	51
	TP 3A	? 003	?		dotty 003	10
					plain ::3	14
					dotty ::3	3
TP 12	PB 12	plain 000	9.4	71	plain 000	52
	PB 12	plain 000	8.7		dotty 000	—
					plain ::3	15
					? dotty ::3	4
TP 15	PB 12	plain 000	7.8	80	plain 000	73
	PB 12	plain 000	7.2		dotty 000	—
					plain ::3	5
					dotty ::3	1
					dotty dubious	1
TP 19	PB 12	plain 000	7.6	183	plain 000	167
	EP 27	dotty 003	8.4		dotty 000	16
TP 29	PB 26	plain 000	8.5	85	plain 000	60
	PB 26	plain 000	7.8		dotty 000	2
					plain ::y	7
					dotty ::y	1
					plain yyy	15
					dotty yyy	—
TP 31	PB 26	plain ::y	9.7	92	plain ::y	92
	PB 26	plain ::y	8.7		dotty ::y	—
TP 104	TP 19	plain 000	6.5	94	plain 000	42
	PB 39	? dotty 123	7.0		dotty 000	4
					plain 123	17
					dotty 123	31
TP 123	TP 19	plain 000	8.5	101	plain 000	41
	TP 31	plain ::y	7.5		plain ::y	60
TP 125	TP 19	plain 000	8.5	87	plain 000	44
	TP 31	plain ::y	7.5		plain 003	43

TABLE 1 (Continued)

Mating no.	Parents			Progeny		
	Source	Score	Size	Total	Score	Numbers
TP 128	TP 31	plain ::y	8.0	98	plain ::y	49
	TP 8	plain ::3	8.5		dotty ::y	4
					plain ::3	42
					dotty ::3	3
TP 139	TP 19	plain 000	8.0	70	plain 000	27
	TP 47	plain 003	5.0		dotty 000	8
					plain 003	7
					dotty 003	6
					plain ::y	17
					dotty ::y	5
TP 201	TP 104	plain 000	10.0	131	plain 000	81
	TP 104	plain 000	9.7		dotty 000	24
					plain 123	19
					dotty 123	7
TP 202	TP 104	plain 000	8.9	171	plain 000	42
	TP 104	plain 000	7.2		dotty 000	88
					plain 123	32
					dotty 123	9
TP 203	TP 104	plain 000	7.4	272	plain 000	147
	TP 104	dotty 000	6.1		dotty 000	49
					plain 123	44
					dotty 123	32
TP 204	TP 15	plain 000	8.4	93	plain 000	93
	TP 15	plain 000	7.2			
TP 206	TP 104	plain 000	9.6	198	plain 000	119
	TP 104	plain 000	9.5		dotty 000	42
					plain 123	37
					dotty 123	—
TP 207	TP 104	plain 000	9.0	76	plain 000	93
	TP 104	plain 000	7.0		dotty 000	85
					plain 123	48
					dotty 123	23
TP 208	TP 104	plain 000	7.9	254	plain 000	132
	TP 104	plain 000	7.6		dotty 000	59
					plain 123	57
					dotty 123	6
TP 209	TP 104	plain 000	7.5	267	plain 000	116
	TP 104	plain 000	5.7		dotty 000	79
					plain 123	34
					dotty 123	38
TP 210	TP 15	plain 000	9.4	59	plain 000	51
	TP 15	plain 000	5.7		dotty 000	5
					plain ::3	1
					dotty ::3	2
TP 211	TP 104	plain 123	9.2	217	plain 123	157
	TP 104	plain 123	7.7		dotty 123	60

TABLE 1 (Continued)

Mating no.	Parents			Progeny		
	Source	Score	Size	Total	Score	Numbers
TP 236	TP 123	plain ::y	8.3	130	plain ::y	122
	TP 123	plain ::y	8.3		dotty ::y	8
TP 237	TP 123	plain ::y	6.6	150	plain ::y	142
	TP 123	plain ::y	5.9		dotty ::y	8
TP 238	TP 123	plain ::y	8.8	92	plain ::y	76
	TP 123	plain ::y	8.1		dotty ::y	16
TP 239	TP 123	plain ::y	7.7	106	plain ::y	100
	TP 123	plain ::y	6.8		dotty ::y	6
TP 240	TP 123	plain ::y	9.4	66	plain ::y	61
	TP 123	plain ::y	7.4		plain ::y	5
TP 241	TP 123	plain ::y	7.0	71	plain 000	35
	MC 3	? plain 00045 hz	7.2		dotty 000	1
					plain ::y	29
					dotty ::y	6
TP 244	TP 123	plain ::y	8.8	22	plain ::y	22
	MC 7	plain ::y	8.2		dotty ::y	—
TP 245	TP 123	plain ::y	10.0	118	plain ::y	69
	MC 7	plain 123	7.7		dotty ::y	49
TP 246	TP 123	plain ::y	7.3	73	plain ::y	38
	MC 7	dotty 123			dotty ::y	35
TP 247	TP 123	plain ::y	9.4	21	plain ::y	7
	MC 3	plain 123	8.8		dotty ::y	14
TP 249	MC 3	plain 00045	7.2	39	plain 000	20
	TP 123	plain ::y	9.0		dotty 000	—
					plain ::y	10
					dotty ::y	9
TP 250	TP 123	plain ::y	6.7	18	plain ::y	6
	MC 5	dotty 123	8.4		dotty ::y	12
TP 251	TP 123	plain 000	7.9	76	plain 000	58
	MC 3	? plain 00045 hz	5.1		dotty 000	1
					plain ::y	16
					dotty ::y	1
TP 252	TP 123	plain 000	8.0	81	plain 000	44
	MC 5	? plain 00045 hz	6.1		dotty 000	—
					plain ::y	36
					? dotty ::y	1
TP 253	TP 125	plain 000	9.8	43	plain 000	23
	MC 3	plain 10005			plain ::y	20
TP 255	TP 125	plain 000	8.8	44	plain 000	
	MC 3	plain 00045	8.1		white shell	15
					cream-brown	16
					plain ::y	13

TABLE 1 (Continued)

Mating no.	Parents			Progeny		
	Source	Score	Size	Total	Score	Numbers
TP 257	TP 125	plain 000	10.0	44	plain 000	10
	MC 7	dotty 123	5.9		dotty 000	13
					plain ::y	17
					dotty ::y	4
TP 258	TP 125	plain 000	9.6	53	plain 000	50
	MC 3	plain 00045	8.4		dotty 000	3
TP 259	TP 125	plain 000	7.1	76	plain 000	72
	MC 3	plain 00005	6.2		plain 100	4
TP 264	MC 3	plain 00045	5.7	28	plain 000	13
	TP 125	plain 003	6.7		plain 00Y	15
TP 266	TP 125	plain 003	7.0	91	plain 003	32
	MC 1	plain ::y	9.9		dotty 003	7
					plain ::y	52
					dotty ::y	—
TP 267	TP 125	plain 003	6.6	48	plain 003	24
	MC 2	dotty 123	5.2		dotty 003	2
					plain ::y	16
					dotty ::y	6
TP 268	MC 3	plain 00045	7.2	31	plain 000	14
	TP 125	plain 003	6.2		dotty 000	1
					plain 003	4
					dotty 003	8
					plain ::y	4
					dotty ::y	—
TP 269	MC 3	plain 00Y	7.1	45	plain 003	13
	TP 125	plain 003	9.6		dotty 003	15
					plain ::y	17
					dotty ::y	—
				109	all 003	58
					all ::y	51
TP 274	TP 128	plain ::y	7.1	91	plain ::y	73
	TP 128	plain ::y	6.8		dotty ::y	18
TP 275	TP 128	plain ::y	8.9	102	plain ::3	27
	TP 128	plain ::y	8.1		dotty ::3	19
					plain ::y	51
					dotty ::y	5
TP 279	TP 128	plain ::3	9.5	71	plain ::3	36
	TP 128	plain ::3	7.5		dotty ::3	17
					plain ::y	17
					? dotty ::y	1
TP 282	H	plain 123	13.4	31	plain 123	17
	H	? plain 123	12.6		dotty 123	6
					123 shz	8
				312	all 123	246
					all 123 shz	66

TABLE 1 (Continued)

Mating no.	Parents			Progeny		
	Source	Score	Size	Total	Score	Numbers
TP 314	MC 6	dotty ? 000	6.4	75	plain 000 etc.	23
	MC 6	plain yyy	6.4		dotty 000 etc.	26
					hz	26
				118	all with	
					pigmented apices	87
					all hz	31
TPR 231	Majorca	plain B100	5.6	213	plain B000	103
					dotty B000	—
	Majorca	plain 003	6.6		plain 003	59
					dotty 003	1
					plain 00:	49
					dotty 00	—
					plain 003 or 00	1

banded, this volume, fig. 2; my Figs. 7, 13) is taken as standard. The ::3, therefore, has bands 1 and 2 more broken up than band 3 on a normal five-banded (compare Figs. 6 and 13), and the form ::y has 1 and 2 well broken, and 3 represented by a yellow band only. Dotty and plain (Cowie, this volume, fig. 2; my Figs. 1, 5) are added as preceding words qualifying the formula. Ideally, the strong solid dark band 3 in the form 003 should be marked as such, but this would require a different type-fount; italics should not be used since these are always reserved for loci and alleles determined by breeding.

Morph is used here in the sense in which it was originally defined by Huxley (1955), as a distinct and genetically-controlled form in a population. It must not be degraded to mean any sort of variety.

DESCRIPTIONS OF SEGREGANT FORMS

The descriptions given below are sufficient to make the different forms, in their typical expressions and in some variants, recognisable. Their exact status, overlaps, and dominance relations are discussed in the next section.

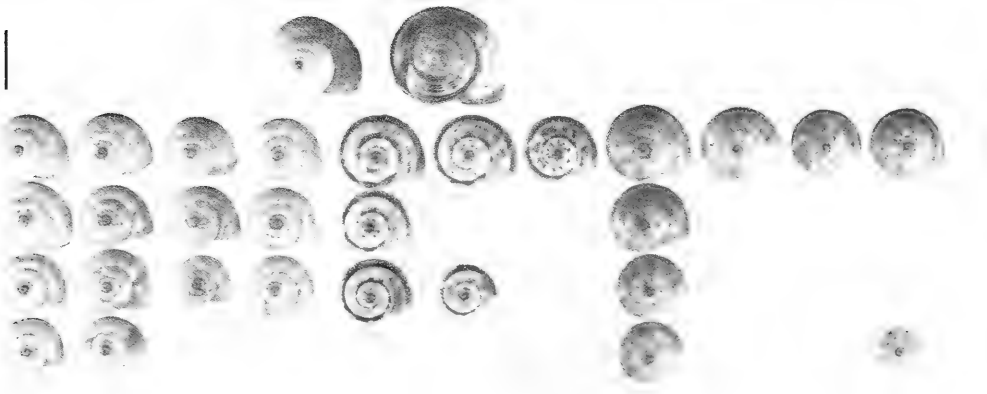

FIG. 1. Mating TP 139, parents and larger shells of progeny. Parents: plain 000, plain 003. Progeny: 1st 3 columns, plain 00y; 4th, dotty 00y. 5th–7th, 003, traces of 1 and 2 increasing to right, the last shell being phenotypically ::3. The low shell in the 6th column is dotty. Righthand 4 columns, ::y, increasing in markings from left to right, the 1st being yyy, the next intermediate ::y, the last rather lightly marked ::y, and dotty, the others in ::y being plain. 2:1:1 ratio. Bar, in this and all other figures through 14, except for Fig. 7, equivalent to 10 mm.

1. *Unbanded, 000* (Cowie, this volume, fig. 2; my Figs. 1–4, 11, 14).

In extreme cases, this is a distinctive form with no bands at all. The full formula is 00000, and in some populations individuals of this formula when young may become mature without adding either yellow or dark bands. In much of the present material, however, traces or more of 5 and sometimes of other bands appear on the last whorl, or earlier in the case of 5 and 4 (my Figs. 2, 3). In mating TP 259, a cross between an unbanded and a 00005, 4 progeny out of the 76 have a dark band 1. Three of these were killed as very small progeny, yet it is evident that the band is confined to the uppermost whorls. In the fourth, which is medium-sized (9.3 mm), it has disappeared before the last whorl formed. In TPR 231 the brown unbanded parent shows band 1 which does not reappear in the progeny (Fig. 11). In PB 12 a parent which from the progeny must be unbanded has a trace of band 2 in brown pigment and was originally scored as ::y. These are the only examples of banding appearing above the periphery in the unbanded form, but the TP 259 mating involves the dubious form 00005.

It is not infrequently found, also, that very young individuals have a few indistinct bandlets of 4 and 5 on the whorl next to the embryonic shell (e.g. TP 104); these disappear, so that the intermediate-sized juvenile is completely bandless. Such transient banding, early or late, is not seen in *Cepaea,* in which the unbanded form is almost always

FIG. 2. TP 269 parent originally scored as 00005. Underside to show acquisition on the last whorl of 4 and 5. Most shells with such formulae behave as fully unbanded 00000. That shown, with 00Y above, is by its progeny banded, not unbanded. Note the muscoid spots scattered all over the surface.

FIG. 3. Unbanded progeny of TP 268, undersides showing continuous variation in the expression of band 5, only yellow-brown pigment at the lefthandmost column, intermediates in the next three, and a black bandlet in the 4th. The 2 shells to the right are 00y (the rest being 000) and show both extremes. There is no sign of either a constancy of 00000 or 00045, or a segregation. This suggests that the 00045 parent is merely a more banded extreme of 00000.

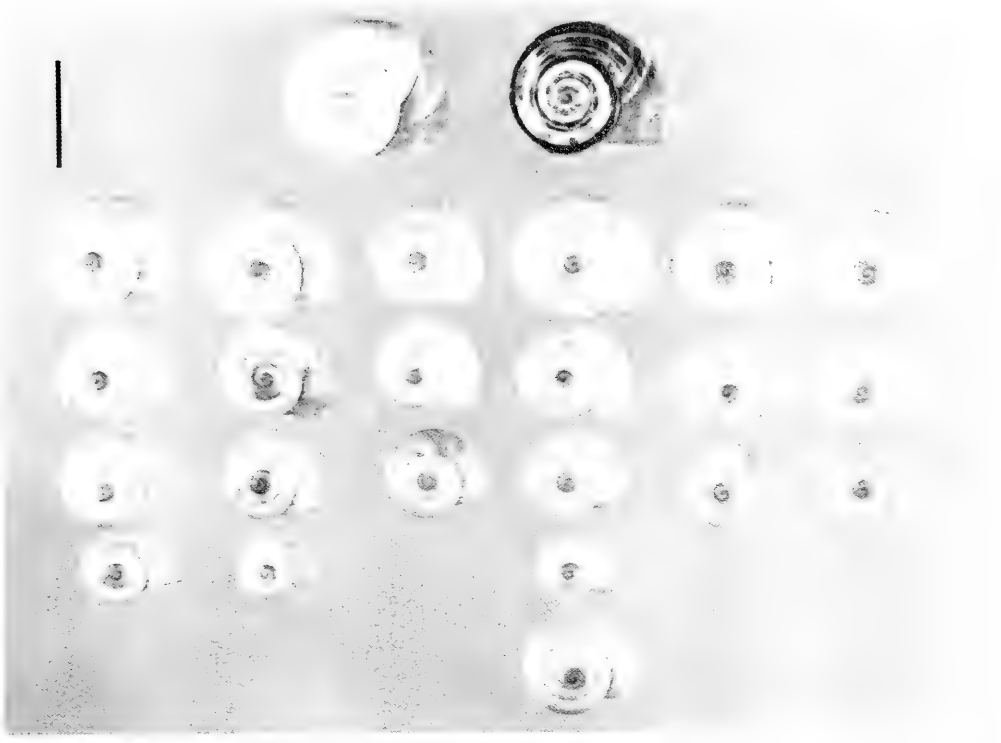

FIG. 4. Mating TP 19 showing dominance of 000 over 003, and a 1:1 segregation of 00y, or possibly 00Y (lefthand 3 columns) and 000 (righthand 3 columns). The unbanded parent is plain, the 003 dotty. The lowermost shell in the progeny is dotty.

clearly distinct from any other (one apparent exception is given by Cain, Sheppard & King, 1968). It is possible that unbanded in *T. pisana* is genetically different from the same phenotype in *Cepaea nemoralis*.

The limits of variation of unbanded are discussed in a later section, since there is a possibility that another form has been included under it. The only matings relating to this other form are TP 249, 253, 255, 258, 259, 264, 268 and 269. Apart from this, there is no question that 000 behaves as a good morph. The formula 000 is used for the morph in general, when modifications are not considered.

2. *00y* (Figs. 1, 4)

It is usual for the unbanded shell, whether white, creamy, cream-buff or buff, to be somewhat browner below the periphery than above, whether or not it is carrying brownish-yellow bands. Above the periphery, in the position of band 3, in some broods there is a pale brownish-yellow band, apparently segregating from a uniformly coloured form. No dark bands appear above the periphery in this form and they are usually reduced or absent below. In other broods the band is faint and difficult to score, and there is considerable doubt as to this form's status (see LIMITS OF FORMS). In a few broods and individuals the band is a noticeably deeper tint, almost brown, and the formula used is 00Y. As shown below, these seem not to have the same genetic basis as 00y.

3. *Dotty* (Cowie, this volume, fig. 2; my Figs. 5–7)

Dotty, first characterized by Cowie (1982; this volume), has a line of dark, usually rounded, dots just above the periphery, in the position of the lowest bandlet of 3 or just below it, occasionally even on the keel of the juvenile shell. Plain, with no dots in this position, and dotty segregate within the unbanded form, with dotty recessive. In my material, what seems to be an identical row of dots appears in the form ::y (Fig. 5), and in 003, ::3 (Fig. 6)

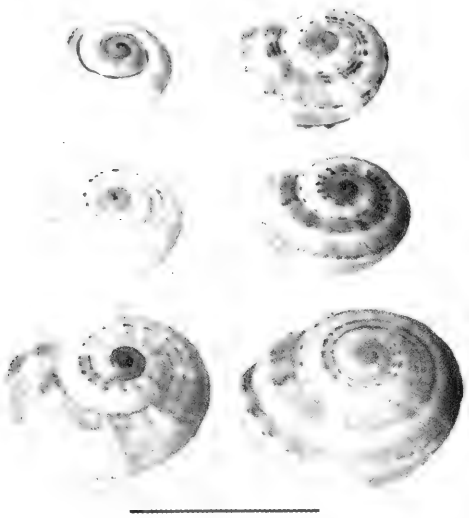

FIG. 5. Selected shells of progeny, mating TP 245, to show plain (lefthand column) and dotty (strong dotty, righthand column) in ::y, in a mating segregating 1:1 for plain/dotty. The dots form a row at or near what would be the lower edge of band 3 if it were present as more than a pale yellow-brown tinge. The top left shell shows band 1 clearly.

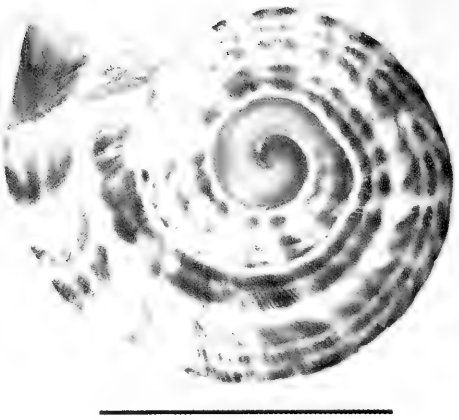

FIG. 7. Dotty in a 5-banded shell. Note the black dots especially at the periphery of the beginning of the last whorl, near where the mouth touches it, and contrast with the dashes or hyphens making up the bandlets of 3, 2 and 1 adapically from them. Bar equivalent to 5 mm.

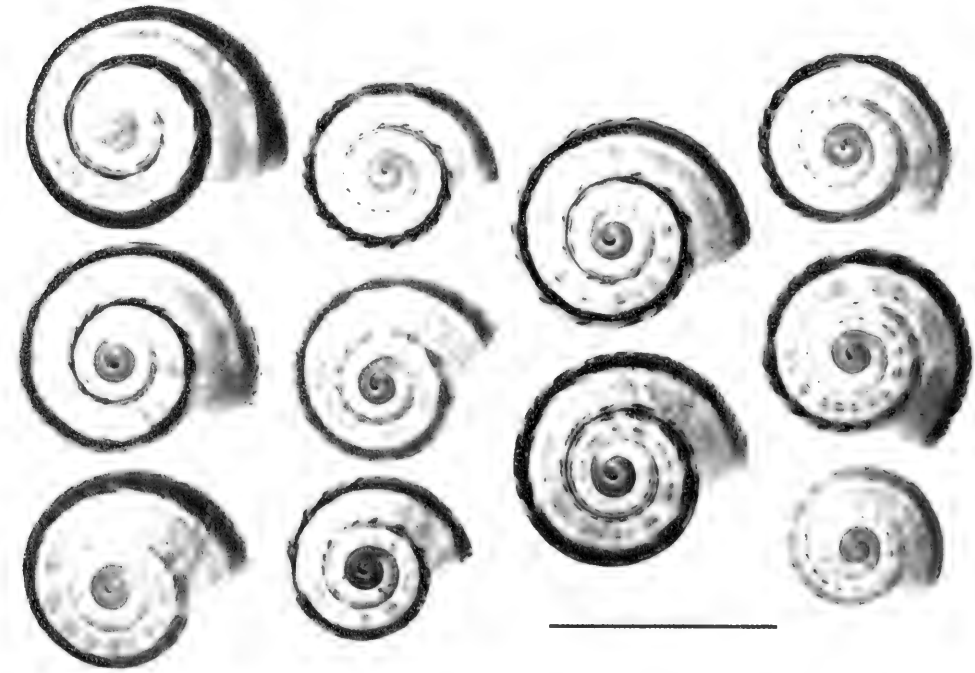

FIG. 6. Possible examples of plain and dotty in ::3; mating TP 279, selected shells. Lefthand column, apparently plain, the feathering of band 3 in the middle shell is not scored as dotty although in some dotty shells the dots may be joined by less pigmented areas to the lower edge of band 3. Middle 2 columns, probably dotty. Compare the upper shell of column 3 with the middle shell in column 1. The lower shell of column 3 is of very dubious score. Righthand column, certainly dotty shells.

and 123 (Fig. 7) (forms described below). Cowie finds that in 000, good Mendelian ratios are produced if all shells, even those with only the slightest indication of dotty, are scored as dotty; I have used the same scoring. Dotty in my material not infrequently falls roughly into three forms, (i) strong, with well-pigmented dots continuing along all the whorls, even on to the last whorl of the adult, (ii) medium, beginning as in the strong form but paling and disappearing when the shell is about half-grown, and (iii) slight, with only a few dots, sometimes very weak, on the uppermost nonembryonic whorl, and fading out very rapidly. In all these forms, dotty is shown very early on in the growth of the shell and can be scored on small shells (3–4 mm diameter). Very few shells have been seen so far in which the pattern develops late (2 are recorded in TP 274).

The shell of *Theba pisana,* unlike that of *Cepaea* and other helicids, shows a class of markings, found also in the helminthoglyptid *Polymita picta,* called muscoid spots, or simply muscoids. These are scattered spots, rarely clustered, formed by small circular or oval semitransparent areas in the shell, seldom pigmented. Often they have a grey tinge because of the colour of the animal showing through the shell, quite different from the yellow-brown of small areas of band pigment. Very rarely one or two may be so situated as to give the impression that the shell is a slight dotty, but this is easily corrected under the binocular microscope, in normally pigmented forms. No distinction could be made in hyalozonate and subhyalozonate.

4. *003* (Figs. 1, 4, 8–10, and, in colour, Taylor, 1895, pl. 2)

This form is highly reminiscent of one found frequently in various species of helicelline snails. The shell above the periphery is very white with no sign of bands 1 and 2 or only one or two dark bandlets. (For simplicity of reference, the form is referred to as 003 irrespective of these bandlets, except, of course, when the limits of variation of forms are being investigated). Band 3 is strongly developed, usually solid black or dark brown, contrasting strongly with the white upper surface. Only rarely is it weak, e.g. in TP 269, discussed below under the unbandeds. Below, 5 is often dark, 4 absent, yellow or dark, so that the form corresponds to the morph 00345 in *Cepaea,* not to the midbanded morph 00300. These forms in *Cepaea,* however, almost never have traces of 1 and 2.

5. *00*: (Fig. 11)

In a single mating, TPR 231, a form of 003 in which band 3 is broken up rather regularly into short sections, and is more lightly pigmented than in most 003, segregates clearly from both 003 and 000.

FIG. 8. Parents of TP 3, a plain 003, and plain ::3. The 003 shows a rather pale band 1 and slight traces of bandlets of band 2. The ::3 has a pale apex, the 003 a dark one but only partly coloured with dark pigment. In the progeny, all 003, 40 are completely 003, 14 have dark traces of 1 or 2, and 5 have both.

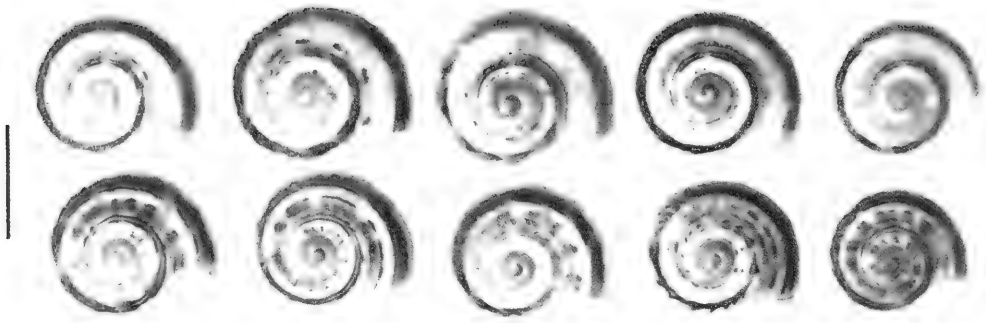

FIG. 9. F_2 progeny of TP 3, selected shells. Upper row, full range of variation in 003, lower row the same in ::3. The actual progeny give a 3:1 ratio 003 to ::3 and the segregation is much better than that shown here, very few of the 003 class having as much extra banding as in the middle shell of the top row. Most are like the two outermost shells of that row.

FIG. 10. Mating TP 267, parents and larger shells of progeny. Parents, plain 003 with trace of 1, and dotty 123, both with a dark apex. Progeny, 1:1 segregation of 003 and ::y, the latter all well-marked except that in the righthand bottom corner, which is intermediate. All progeny with dark apices. The 003 parent carries ::y recessive to it. Both 003 and ::y are dominant to 123.

6. *::3* (Figs. 8–10, 12)

In this form, rather or very interrupted bandlets are present on 1, 2, or 1 and 2, but 3 is markedly stronger, as in 003, although not usually as much so as in that form.

7. *::y* (Figs. 1, 10, 12)

In contrast to 003 and ::3, in this form band 3 is much weaker than 2 (or 1 and 2), at least in the early stages of growth, when it first appears as a yellow band. Although in some

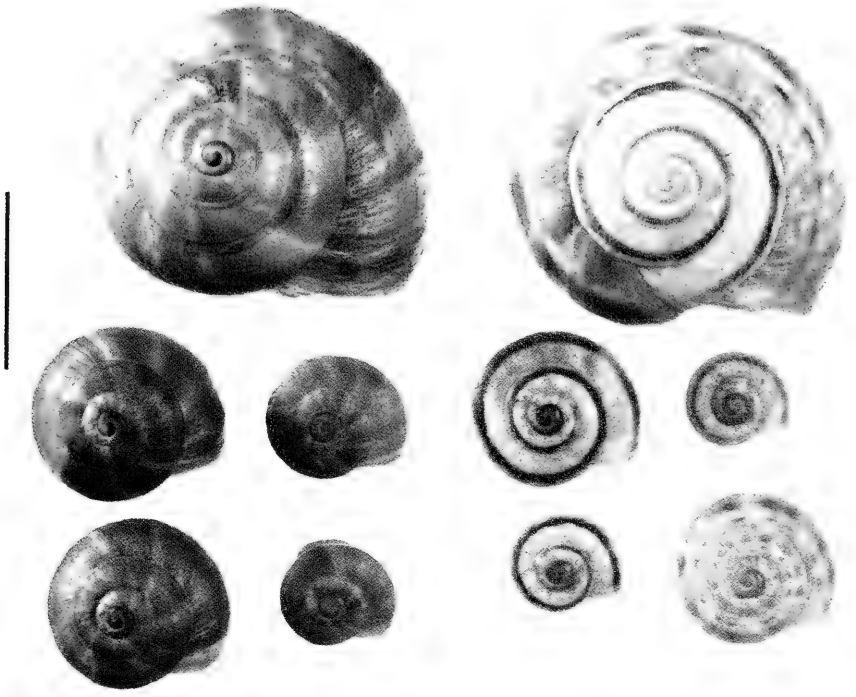

FIG. 11. Mating TRP 231, parents and a selection of shells of progeny to show the 2:1:1 ratio of brown 000, 003 and 00: (two extreme shells shown, righthandmost column). Parents, plain B 000 with trace of 1 and dark apex, plain 003 with pale apex. Almost all the 003 and 00: have dark apices as here, the browns, also as here, show a 1:1 segregation of dark apex (lefthandmost column) to pale (next column). Only a single 003 shell is dotty (not shown here), all the rest are plain.

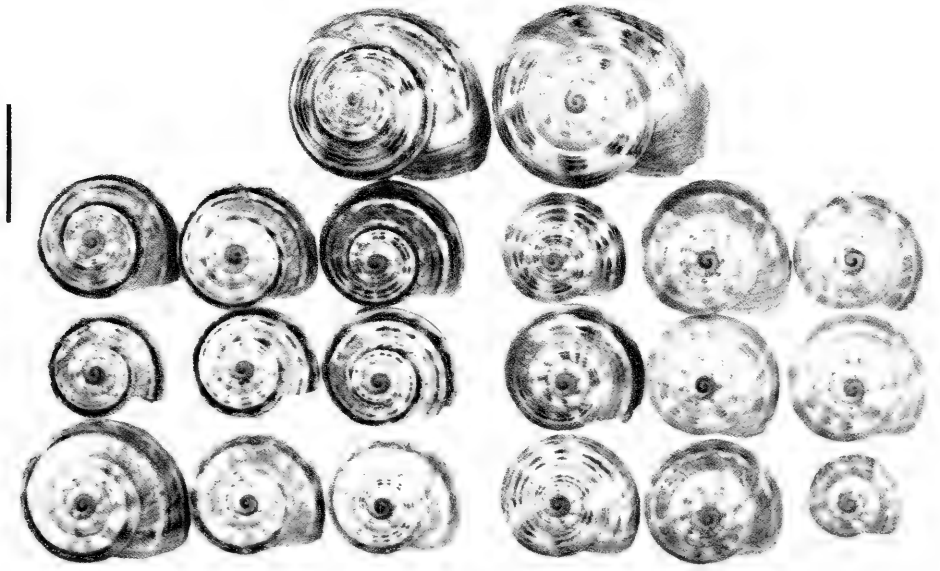

FIG. 12. Mating TP 128, parents and larger progeny. 1:1 segregation, ::3 (3 lefthand columns) and ::y (3 righthand columns). In the lefthand half, band 3 is accentuated even on the earlier whorls, while in the righthand half it is less well pigmented than 1 and 2, although developing later. Scoring is therefore easiest on the earlier whorls of the shell, and there is no difficulty in recognising a segregation. Parents, plain ::3, dark apex; plain ::y, apex just dark. All apices of juveniles with some dark pigment.

matings it seems fairly uniform in expression, in others it is rather variable, ranging from 123 through ::y to yyy, but still segregating from ::3 and 003, and, with some overlap, from 123. In the fully ::y phenotype there is much variation in the bands shown, either 1 or 2 or both being with black markings. For simplicity, the single formula ::y has been used throughout except for special purposes.

8. *123* (Cowie, this volume, figs. 1, 2; my Figs. 7, 13)

In this form, the bands are usually developed below as well as above (4 is sometimes late or only yellow) and in general it agrees with Cowie's dark 12345. The detailed characters (numbers and degree of interruption of bandlets, degree of pigmentation etc.) differ, often rather noticeably, from colony to colony. While this may be due to nothing more than slight variations in the genetic background from place to place, it is quite possible that at present, several forms differing in major genes are included here. The different degrees of dark pigmentation (Cowie's dark 12345, intermediate, and yellow) may well be genetically controlled. His yellows, with only a few traces of dark bands 1, 2 and 3, or even 1 and 2 only, on the highest whorls, but with strong yellow bands, are very similar to the yyy given just above as an extreme of ::y. As this latter form does not seem to occur at Tenby, this is almost certainly convergence in phenotypic expression, and it is better to leave Cowie's form as yellow 12345, until further work on these forms can be done.

9. *Hyalozonate (hz), subhyalozonate (shz)* (Figs. 13, 14)

The complete abolition of all banding pigment in *Cepaea* results, in banded morphs, in shells with glassy transparent bands, the transparent areas marking the parts of the shell modified to receive the banding pigment. Often, but not always, the ground colour of the shell (brown, pink or yellow) is also abolished, resulting in a pure white shell with glassy bands (or in unbandeds a plain pure white shell). Dr. C. B. Goodhart very kindly showed me such shells of *Cepaea nemoralis* from Cambridgeshire which were hyalozonate except for faint clouds of banding pigment at the ends of the bands nearest the mouth. The pallid form from St. Ives, Cornwall (Fig. 13), agrees at first sight with the hyalozonate condition, but closer inspection shows that there are slight traces of pale brownish pigment all along the bands. It can therefore be called subhyalozonate; the pigment distribution is very different from that of Dr. Goodhart's *Cepaea,* which could also be brought under the same term.

FIG. 13. Mating TP 282, parents and larger shells. Parents, one plain and one probably plain 123 dark apex. Progeny, 3:1 segregation of 123 and subhyalozonate, the latter with colourless apex and only faint yellow pigment in patches along the bands which are mostly transparent; apex colourless, shell dead-white.

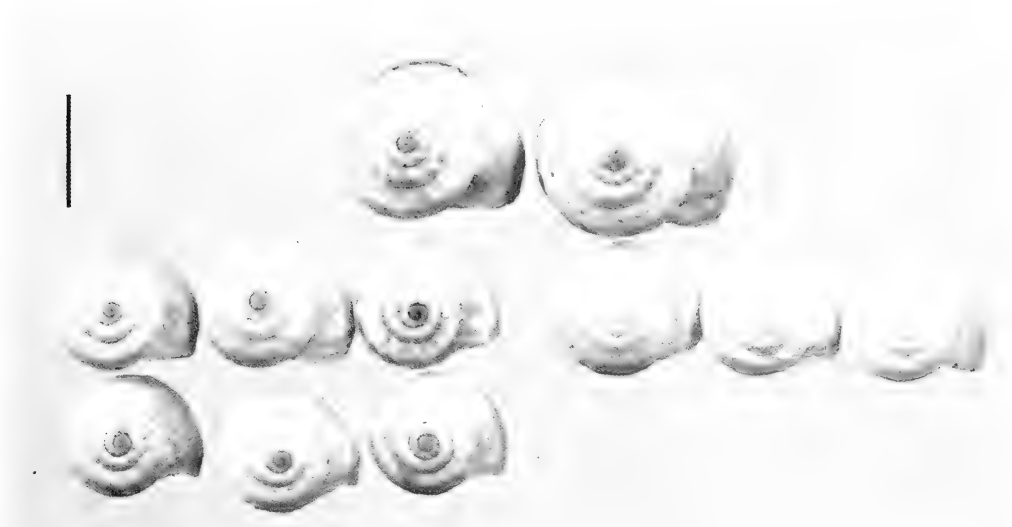

FIG. 14. Mating TP 314, parents and larger progeny. Parents to left, plain yyy; to right, dotty 00y, developing to very lightly-marked 023. Progeny, 3:1 segregation of 00y, and true hyalozonate 000 with colourless apex and a few traces of bands (beneath the shells) completely transparent, with no pigment. The hyalozonates are dead-white, the 00y creamy. In the latter, a few shells have the yellow band very strong, 00Y (e.g. the righthandmost of the 00y) and it is not certain that the 00y class may not be a very lightly marked form of ::3. (These larger 00y shells are dotty, but there is a segregation in the class.)

Dr. Cowie tells me he has seen true hyalozonates from Mediterranean localities; and the dead-white unbanded shells in TP 314 (Fig. 14) are probably hyalozonate unbandeds.

10. *Shell colour*

Shells suffused with a fine claret colour have been bred by Dr. G. Lewis (personal communication). These are a distinct morph, pink. Rarely, among bred material which is carrying the allele for a pink mouth (Cowie, this volume), one gets a few badly-grown juveniles in which the pink colour seems to be laid down prematurely, giving the shell a red tinge. Such specimens show abnormally rugose shells, and may well occur only in breeding-boxes, not in the wild.

The general colour of the shell, when not pink, varies from white (a little more creamy than the dead-white of hyalozonates) through cream to buff and brown. In fivebanded shells with strong dark or yellow bands, the ground colour may be obscured and very difficult to score. In the form 003 it is normally white above, probably to contrast with the dark band 3. In many unbandeds it is distinctly browner below the periphery than above; the difference is much more marked than that occasionally seen between the shades of colour above and below the periphery in *Cepaea*. Because of these complications, ground colour is often not scorable with certainty, and little attention has been paid to it here. Almost all the shells in these matings vary from medium white to creamy buff above.

The principal exception is of brown shells in TPR 231 (Fig. 11) from Majorcan juveniles, bred by Dr. R. H. Cowie. These are tan-coloured, a strong warm medium brown, and segregate clearly from the pale-shelled bandeds in their mating (creams and cream-buffs). Unfortunately, other matings of similar forms failed.

The symbols B, P and A (for *alba*) will be used for the brown, pink and dull white to cream-buff shades respectively.

11. *Apex colour* (Cowie, this volume, fig. 9; my Fig. 11)

The apex of the shell (the first whorl of the embryonic shell) varies considerably in degree of pigmentation, from none through various shades of brown to purple-black. Colourless or near-colourless apices have been seen only in hyalozonate and subhyalozonate. Pale ones are light brown, dark ones have at least part of the apex very darkly pigmented; if not uniformly coloured, they

usually have the very dark pigment restricted to a band broad at the very tip and narrowing progressively, confined to the outer side of the upper whorl. In TPR 231 there is a good segregation with very few of doubtful score, of dark apex in 003, pale in 00:, and of both, 1:1, in tan 000. Cowie (this volume) finds a similar segregation with dark recessive to pale. In some of my matings scoring is much more difficult and there may be continuous variation, as discussed later.

Apex colour can be scored with ease only in fresh shells from which the animals have been completely extracted. In old shells there is often external abrasion which whitens the apex. There may also be some paling by internal accretion in large adults. In dead shells, there is often some organic matter left in the top of the spire, which blackens it.

GENETICS OF FORMS

The unbanded form is dominant to 003 (matings TP 19, 125), ::3 (TP 12, 15, 210), ::y (TP 29, 125, 251, 252) and 123 (TP 201-3, 206-9), this last confirming the findings of Cowie (this volume). This agrees with the situation in *Cepaea*; the relation of 000 to 003, the only two forms bred so far, in the helicelline *Cernuella virgata* (Da Costa), however, is the opposite (Cain, in preparation).

Form 003 is dominant or epistatic to ::3 (TP 3), and to 123 and ::y (TP 267).

Form ::3 is dominant or epistatic to ::y (TP 279).

Form ::y is dominant or epistatic to 123 (TP 267).

It appears, therefore, that we have a hierarchy as in *Cepaea,* in the order, beginning with the top dominant, 000, 003, ::3, ::y, 123. As in *Cepaea,* the fewer the bands the higher the dominance rank except for ::y, a form which has no analogue in *Cepaea.* It is not possible from these matings to distinguish between dominance and epistasy; at present, there is no evidence that the forms just listed are not alleles at the same locus.

Plain is dominant to dotty in unbanded (TP 201, 3, 6, 8), and must be at a separate locus from unbanded/banded, as shown by Cowie (this volume), and by the relevant present matings.

Apex colour, from PB 12 and TP 231, appears, as in Cowie's material (this volume) to be a separate locus with pale dominant to dark, closely linked to the banded/unbanded locus; this is the only certain evidence so far of linkage in shell characters.

Subhyalozonate is known so far only from St. Ives. Mating TP 282 shows the near-abolition of the banding and shell pigment to be recessive to normal pigmentation as is the case with hyalozonate in *Cepaea nemoralis.* Presumably it is at a different locus from the various banding forms, since in TP 282 it is segregating within the form 123. In TP 241, 251 and 252, what seems to be true hyalozonate disappears when crossed even to normal creamy unbanded shells, the characteristic dead-white being lost completely. Presumably it is recessive, too.

LIMITS OF FORMS

Unbanded, 000

One group of matings, TP 249, 253, 255, 258, 259, 264, 268 and 269, was set up to test the genetics of individuals with formula 00045 and 00005. In matings TP 258 and 259 these were crossed with ordinary unbandeds, and gave only unbandeds, with some adventitious banding as described above under 000. In TP 268, a cross with 003 gives a 2:1:1 ratio of ordinary unbanded, 003, and ::y. Fig. 3 shows the range of banding variation below the periphery in the unbandeds, there being no banding above it. This is within the variation of adventitious bands in ordinary unbanded juveniles of comparable size. While TP 258 and 259 could be interpreted as showing the dominance of ordinary unbanded over the 00045 form, this is contradicted by TP 249 and 268 in which no unbanded is present in the parents, only 00045, yet ordinary unbandeds appear. The form 00045 must therefore be only an unusually banded 00000. The same argument holds for TP 253 and 255 in which a proportion should be 00045, but is not. In TP 264, a replicate of 268, only ordinary unbandeds appear. A few have a faint indication of 5, and 2 have 5 pigmented and 4 indicated, again as in matings of unbandeds; this confirms the results of TP 268, but with a 00045 parent homozygous for unbanded.

However, in TP 269, apparently a replicate of 264 and 268 when set up, only banded progeny appear. The 00005 parent was scored as such at 7.2 mm; but it was noted later to be 00y, with y strong and brownish on

TABLE 2. Detailed scores of larger progeny of TP 314, omitting hyalozonate. There is difficulty in scoring 00y.

	Phenotype	Numbers
(a) Plain	000?	4
	00y	6
	00Y	2
	00:	9
	:::	2
(b) Dotty	000	7
	00y	4
	00Y	7
	00:	8
		49

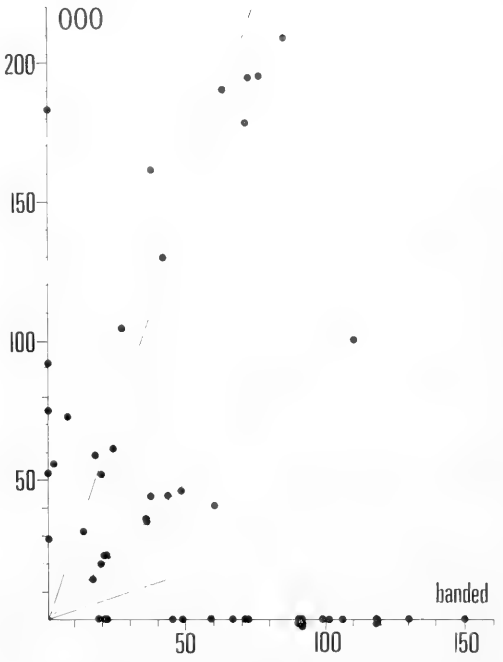

FIG. 15. Discriminant diagram for unbanded against all banded forms in progeny. Two scores are off the diagram; 000 0, banded 217, and 000 0, banded 312. Vertical axis, actual numbers of unbandeds; horizontal axis actual numbers of bandeds. The lines drawn in, from the vertical axis clockwise, are for ratios of unbanded to banded, 1:0 (vertical axis), 3:1, 1:1, 1:3 and 0:1 (horizontal axis).

the early whorls. The clearly marked band 5 was lost as it grew and as a large juvenile it was unbanded. The yellow band in position 3 at this stage became a little paler, then darkened on the last whorl, acquiring black bandlets. Bands 4 and 5 appeared first in yellow, then darkened like 3 on the last whorl (Fig. 2). Finally, traces of dark marks appeared close to the mouth on band 2. The 003 parent is known from its pedigree to be heterozygous for ::y. The total progeny from this mating, including very small shells which seem in this case to be scorable easily, is 58 003: 51 ::y, a good approximation to the 1:1 ratio expected if only two classes, and one of them ::y, segregate in the offspring. It follows that the apparently 00005 parent must either be homozygous for ::y, or heterozygous for ::y and some form recessive to both it and 003, or wholly recessive to both 003 and ::y. The only form so far found to behave in a suitable way is 123. The parent certainly resembles neither it nor ::y. It differs from the corresponding parents in the other matings only by the rather emphatic colour of band 3, and should have been scored as 00Y. In TP 314, one parental shell with no dark bands but with a pale yellowish band, broken up into flecks, in the position of band 3, scored originally as 00y00 developed into a very lightly and palely marked 02345. The other, scored originally as 00yy5 but now as yyy above at that size, hardly increased its markings until the last whorl, and even then only by dusky traces of 3 and 4. The scores of larger progeny, except for the segregant hyalozonates, are given in detail in Table 2, and the parents and largest shells are seen in Fig. 14 (including hyalozonates). Plain/dotty seems from Table 2 to segregate independently of the other variation in the progeny and can be disregarded. These phenotypes seem to be a continuum, in which case both parents must have been genetically banded. Alternatively, the phenotypes can be grouped into 21 000 + 00y and 28 with stronger markings, a probable 1:1 ratio. In this case, one of the parents must be genetically banded. There is no further information available on these very palely-marked phenotypes, which may well constitute a distinct form.

These are the only examples in the present material of an unbanded (scored on the medium-sized juvenile) being genetically banded. Probably all forms scored as 00045 and 00005 when small need further examination. For the present (and except for TP 269) they have been left with the unbandeds.

The discriminant diagram (Fig. 15) for unbanded against banded shows a good

clustering of progeny ratios around the expected ratios 1:0, 3:1, 1:1 and 0:1 with none approaching 1:3.

A small number of matings of 000 and 000 give only a few bandeds, markedly less than the numbers expected; these are seen in the diagram very close to the vertical axis. It is not certain whether the deficiency is due to low penetrance or poor survival of the banded class.

Apart from the 00Y parent of TP 269 and one or both of the parents of TP 314, which, as just shown, must be banded, there appears to be no difficulty in scoring the unbanded form. Indeed, even in random samples, unbanded shells when viewed from above (and often from below also) are readily separable from the rest in the light of breeding experience.

00y

It is clear from matings TP 12, 15, 29, 201, 202 and many others that 00y is a modification of unbanded (Table 3, compare Table 1). The combined scores for 000 and 00y give the most frequent class in several 3:1 seg-

TABLE 3. Scores of 000 and 00y in unbandeds. Parental formulae 000 and 00y contracted to 0 and y respectively.

Mating no.	Parents	Progeny 000	Progeny 00y	Progeny Total	Ratio	Expected 00y
PB 12	0 × 0	149	39	188	3:1	47.00
PB 26	y(× ::y)	19		19	1:0	
TP 12	0 × y	23	29	52	1:1	26.00
TP 15	0 × 0 ?	16	28	44	? 1:3	33.00
					or 1:1	22.00
TP 19	0(× 003)	92	91	183	1:1	91.50
TP 29	y × y	19	43	*62	1:3	46.50
TP 104	0?(× 123)	12	25	*37	? 1:3	27.75
					or 1:1	18.50
TP 123	0(× ::y)	15	26	41	? 1:3	30.75
					or 1:1	20.50
TP 125	y(× ::y)		44	44	0:1	
TP 139	y(× 003)	6?	13	19	? 0:1	
TP 201	0 × 0	37	6	43	3:1	10.75
TP 202	0 × 0	15	23	38	? 1:3	28.50
					or 1:1	19.00
TP 203	y × y ?	128	50	178	3:1	44.50
TP 204	0 × 0	93		93	1:0	
TP 206	y × y	41	84	125	? 1:3	93.75
					or 1:1	62.50
TP 207	y × y	44	48	92	1:1	46.00
TP 208	y × y	29	44	73	? 1:3	54.75
					or 1:1	36.50
TP 209	y × y	5	19	24	1:3	18.00
TP 210	y × y ?	10	46	*56	1:3	42.00
TP 241	(hz)(× ::y)	14	16	*30	1:1	15.00
TP 249	y(× ::y)	20		20	1:0	
TP 251	0(× hz)	56	3	59	3:1	14.75
TP 252	y(× hz)	34	10	*44	3:1	11.00
TP 253	y × ? y	4	19	23	1:3	5.75
TP 255	0 × 0	31		31	1:0	
TP 257	y(× 123)	11	12	*23	1:1	11.00
TP 258	y × y ?	35		35	1:0	
TP 259	y × 0	31	4	*35	3:1	8.75
TP 264	0(× 003)	13	15	28	1:1	14.00
TP 268	y(× 003)	2	13	15	1:3	11.25
		1004	750	1754		

*Scores dubious, probably continuous variation.

regations, with considerable accuracy. It is very difficult, however, to say what is the genetic status of this modification. The band is often faint and variable, and the presence of a segregation is very doubtful. In the present matings, shells are scored as 00y if there seems to be any trace of the band at all. The results are highly contradictory.

If there is doubt about the scoring of any character, then it is possible that some proportion of parents is mis-scored, and it is best to consider primarily the progeny, using them, since they are numerous, to judge the accuracy of scoring of the parents which can only be two. In TP 29, 209, 253 and 268 (Table 3) there are approximations to a 3:1 ratio with 000 recessive, but scoring is difficult, and at least in TP 29 there may be a continuum. In PB 12, TP 201, 203, 209, 210 (possibly a continuum), 251 and 252, there appears to be the reverse ratio, with 00y recessive. In TP 204, from two 000 parents, only 000 appear; at the very most 2 out of 93 offspring could just possibly be 00y. In TP 258 only 000 appear, but the parents seem both to be 00y. In TP 125 and 139 all the offspring are apparently 00y and in both, the unbanded parent is 00y. These matings suggest strongly that there is a genetic component, but that mis-scoring is frequent. It is not possible to determine whether superpenetrance or subpenetrance of either form is responsible. The largest numbers are available in TP 203, and give an excellent agreement with 00y being recessive (observed, 50; expected, 44.50). The largest number for the opposite ratio, however, (TP 206, observed 41; expected 31.25) is nearly as convincing. If either of these results is due to incorrect penetrance, the discrepancy must be about 25%, and very little reliance can be placed on any interpretation.

A heterogeneity χ^2 on all matings with a total of unbandeds greater than 20 merely confirms that there is genuine heterogeneity. The discriminant diagram (Fig. 16) contrasts sharply with that for 000 and bandeds in which the points are clearly associated with the ratio lines.

Dotty in unbandeds and 00045

In matings TP 204, 259 and 264, all the offspring are plain unbandeds, as are the parents. In TP 258, with plain parents, there is a segregation of plain and dotty approximating to 3:1, which confirms Cowie's finding that plain is dominant to dotty. The diagram for all

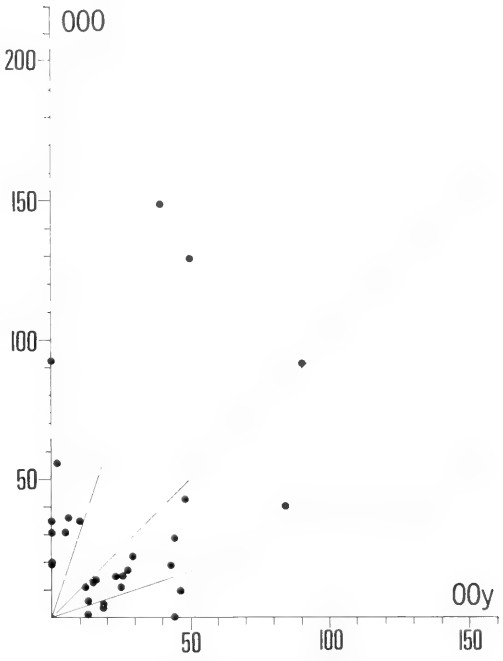

FIG. 16. Discriminant diagram for 000 against 00y in unbanded progeny. Conventions as in Fig. 15.

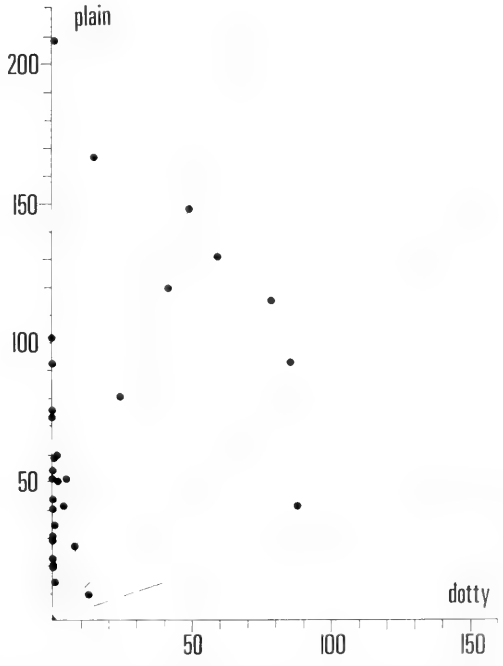

FIG. 17. Discriminant diagram for plain against dotty in all unbanded progeny. Conventions as in Fig. 15.

segregations (of dotty) in unbanded (Fig. 17) is in good agreement with this except for a single mating, TP 202, approximating to a 1:3. On checking the segregations observed within the unbanded progeny against parental scores, one finds only 6 matings (TP 19, 104, 202, 203, 207 and 209) with a discrepancy. In TP 19, either the 003 parent is not dotty and this is a 3:1, or it is dotty and, since the recessive class is highly deficient (observed, 16; expected 45.75), this is really 1:0 with a small amount of superpenetrance, about 10%, in the heterozygotes. In TP 104, the same argument applies to the dotty 123 parent. In TP 202, a really aberrant ratio appears to contradict all the rest of the evidence; either both parents are really dotty but there is a massive subpenetrance of the homozygote (over 30%) or one parent is dotty and this is a 1:1, perhaps simply affected by sampling error. In TP 203, the progeny agree excellently with a 3:1 ratio, and the dotty parent must therefore be showing superpenetrance. In TP 207, with a good 1:1, on the contrary one parent must be showing failure of penetrance. The same appears to be true of TP 209. Out of 31 matings (Table 1) producing unbandeds, avoiding the dubious TP 314, we find among the parents 49 plain 000, 3 plain 003, 3 plain ::y, and 1 dotty 123 for which there is no reason to doubt their scores; and 3 plain 000, 1 dotty 000, 1 dotty 003, and 1 dotty 123 which require a change of phenotypic score because of their progeny. Of the unbanded parents (since dotty in bandeds requires further discussion below), 3 plain 000 are genetically dotty, 1 dotty is genetically plain, an overall error of about 8.5%, the particular errors being in both directions. This agrees well with Cowie's findings (this volume) on material from a different locality. (It is possible, however, that the estimate of error may be biased; the only 000 dotty is not dotted genetically, and the error in this direction could be vast.)

It is noticeable from Table 1 that in a number of matings of plain with plain, only a very few dotty shells appear in the progeny—e.g. TP 19 discussed above, and more extreme ones such as PB 12 (observed, 1; expected 52.25). These are close to the vertical axis in Fig. 17. Since they have dotty at all, they cannot belong to the purely homozygous plain class, and all depart significantly from 3:1. A heterogeneity χ^2 for them and those close to 3:1 is very highly significant. If they are examples of superpenetrance in a 1:1 ratio of plain homozygotes to plain/dotty heterozygotes, it varies in incidence from about 10 to about 0.5%. If they are deficiencies due to lack of viability, this must be really massive in 8 matings out of 21. Perhaps superpenetrance is the most likely hypothesis as yet.

The discriminant diagram (Fig. 17) shows a good approximation to the expected ratios, but with a number of symbols close to the vertical axis, representing segregations very deficient in dotty. Since no dotty $\times$ dotty matings were set up, the ratio 0:1 is not represented.

Dotty in bandeds

Two classes of matings can be considered, (i) those in which both banded and unbanded are segregating, so that a comparison of the ratio of plain to dotty can be made in both, using that in unbandeds as standard, and (ii) those in which there are only banded progeny. One can also distinguish within bandeds the ::y form, in which the only dark markings on band 3 in the juvenile shell are the dots when present (Fig. 5), and the other banded forms in which the expression of band 3 may interfere with the scoring of dotty (Figs. 6, 7). The lowermost bandlet of 3, when developed, may begin as short dark dashes approximating to dots. If it is more continuous it may overlap and incorporate the dots in some proportion of dotty shells (the solid band 3 in the 003 parent in Fig. 8 appears wide enough to do so). Although Fig. 7 suggests that their position is below that of the lower edge of 3, there may be considerable variation in this respect. Material from Tenby shown me by Dr. Cowie has some shells which could be scored as dotty 123. As none of his material corresponds exactly to ::y, and scoring in 123 is difficult, dotty in banded has not been investigated by him at Tenby.

1. Plain/dotty in unbandeds and bandeds segregating together

Although some of the segregations in this class of matings give good approximations to the standard Mendelian ratios, considering the smallness of the numbers of banded shells in some progeny (e.g. for 3:1, TP 201, 208; for 1:0, TP 123), there are often striking discrepancies between the ratio in unbandeds and bandeds of the same brood. In TP 104, if the parental scores are right, the

ratio in bandeds is probably correct (1:1), that in unbandeds (3:1) certainly wrong; if the dubiously dotty parent is genetically plain, then it is the ratio in the bandeds that is wrong. In TP 202, already noticed as difficult to interpret on its plain/dotty ratio in unbandeds (44:88), the ratio in bandeds (39:9), on the contrary, agrees well with the expected 3:1 if the parents are correctly scored. In TP 203, the 3:1 ratio in unbandeds agrees well with the parental scores, but in the bandeds, all of them 123, a good approximation to 1:1 appears. The numbers of progeny are quite large in these matings.

The discriminant diagram (Fig. 18) for plain/dotty in bandeds segregating from unbandeds shows less clear agreement with ratios than in Fig. 15, largely because of smaller numbers. As in Fig. 17, there are a number of symbols, representing broods with deficient dotty, close to the vertical axis.

Omitting TP 15 and 210 as with too few bandeds, there are from χ^2 tests 14 matings without and 10 with a significant discrepancy in plain/dotty between unbandeds and bandeds at the 1 in 20 level of probability. The expected number of matings showing a discrepancy by chance at this level is only 1.2, and this departure from expectation is itself highly significant. Of the 10 discrepancies, 5 are with an excess and 5 with a deficiency of dotty. In no case of segregation within the bandeds (TP 29, 139, 268; TPR 231) is there a discrepancy in plain/dotty between the banded forms involved. Classifying by the banded forms, we find (Table 4), as far as the rather limited evidence goes, no systematic tendency in the sign of the discrepancies in plain/dotty between unbandeds and bandeds, nor any indication that they differ between forms with band 3 dark (003, ::3, 123) and that with it only yellow (::y). Either, therefore, the different types of errors involved in scoring dotty in ::y and those affecting its recognition in forms with 3 dark are approximately equal and of much the same variance, or dotty is not subject to different types of scoring error in different forms.

A χ^2 on all segregations of plain and dotty, approximating to a 3:1 ratio, within all unbandeds (whether themselves segregating bandeds or not) shows highly significant heterogeneity. The matings departing from a 3:1 ratio, already noticed as clustering close to the vertical axis in Fig. 17, may well be not true segregations but examples of slight superpenetrance. However, they include TP 104, in which there is an excess of dotty in the segregant bandeds, all 123, significantly different from the deficiency in unbandeds. If penetrance of dotty were much greater in bandeds than in unbandeds, one would expect to detect the difference in other matings. The remaining ones with a deficiency of dotty (PB 12, TP 29, 241, 251) show no discrep-

TABLE 4. Discrepancies in plain/dotty within the banded forms compared with segregant unbandeds in the same progeny.

Form	More dotty	No discrepancy	Less dotty	Total
::y	2	10*	1	13
003	1	3**	—	4
00:		1		1
::3		2		2
123	3	1	4	8
	6	17	5	28

*As there is no significant difference between ::y and 003 in TP 268, the ::y should perhaps be scored as with too many dotty.

**One is doubtful (see numbers in Table 1 for 003 against ::y in TP 139).

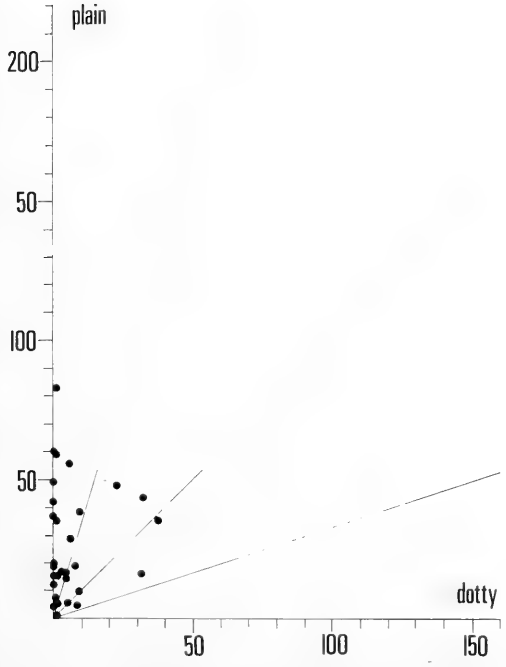

FIG. 18. Discriminant diagram for plain against dotty in bandeds segregating in progeny from unbandeds. Conventions as in Fig. 15.

ancy between bandeds and unbandeds in this respect.

2. Plain/dotty in bandeds only

As in the preceding section, several matings show good approximations to the standard Mendelian ratios; there are some discrepancies (Fig. 19) though nothing like those in 000 and 00y (Fig. 16); and there are again a number with a deficiency of dotty falling close to the vertical axis. Taking all the matings concerned and scoring segregant forms within bandeds independently, there are, of those showing ratios from near to 3:1 to nearly 1:0, 12 with a deficiency of dotty and 4 with an excess; in those approximating to 1:1 it is 2 and 4 respectively. The ratio of deficits to excesses gives no indication of varying with respect to banding form, the actual numbers being for 003, 4 deficits, 1 excess; for ::3, 2 and 2; for ::y, 11 and 3; and for 123, 0 and 2. In 6 broods there are segregations within the banded class (TP 3B, 128, 267, 269, 275 and 279) of which in 2 (TP 269, 275) the banded forms differ significantly in plain/dotty ratio. As in the discrepancies between plain/dotty in

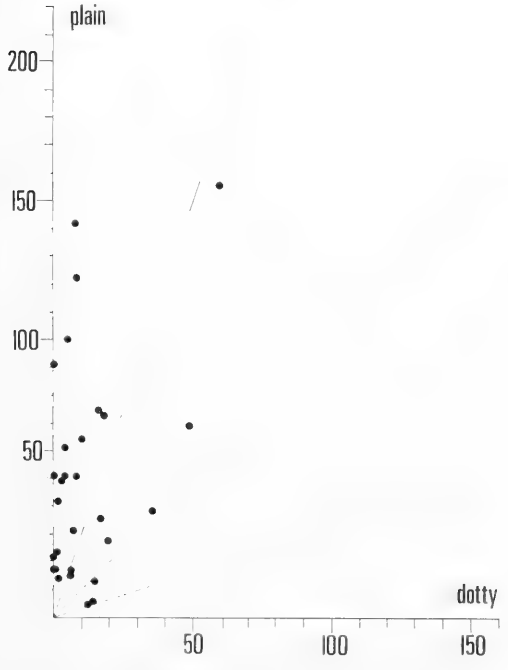

FIG. 19. Discriminant diagram for plain against dotty in progeny consisting only of bandeds. Conventions as in Fig. 15.

unbanded and banded (TP 104, 202, 203, 207, 209, 249, 257, 268), in one form there is an approximation to a 3:1 ratio, in the other to a 1:1 (although between unbandeds and bandeds in TP 206 and 208, there is a significant difference between a 3:1 ratio and one approximating to 1:0). There are hardly enough matings for a test of close agreement with Mendelian ratios, but the figures certainly suggest that agreement is often good.

Parental mis-scorings in plain/dotty

Every mating can be used to look for discrepancies between the phenotypic scores of the parents as plain or dotty and their genetic constitution as revealed in the offspring, except TP 3B, the exact parents of which are not known. Where the plain/dotty ratio differs significantly between segregant forms in the progeny, different assessments of parental scoring are produced, as with TP 104, 202 and 203 discussed above. Where the two parents differ in banding form but not in plain/dotty score, yet one must be wrong, it is not possible to say which. For example, in TP 245, the apparent 1:1 ratio in plain/dotty in the progeny shows that either the plain ::y or the plain 123 must be dotty, but one cannot go further.

In Table 5, all the parental scores for plain and dotty are shown, classified by types of matings and by parental forms. The queried figures are necessitated by the unresolved doubts just mentioned—thus in TP 245 one plain ::y and one plain 123 may each be either rightly or wrongly scored. These doubtful cases, fortunately, are comparatively few, and are disregarded in what follows. There are no marked differences in the incidence of error between matings with unsegregating and segregating progeny. There is a strong hint that scoring in 123 is much less reliable than in the other forms. Also, provided the estimates of errors can be trusted (but see the comment under *Dotty in unbandeds and 00045* on unbanded dotty), dotty is more often genetically plain than plain is genetically dotty, i.e. superpenetrance of dotty is more common than subpenetrance. These results from genetically tested individuals are probably more cogent than those given above from the consideration of the progeny.

It seems, then, that there is more incorrectness in the score of plain/dotty than in that for unbanded/banded, though much less than for 000/00y. In view of Cowie's findings (this volume) and the results presented here, there

TABLE 5. Correctness, classified by parental form, of parental phenotype score as judged from the progeny. TP 3B omitted as its exact parental scores are unknown.

Parental form	Plain			Dotty			
	Right		Wrong	Right		Wrong	
A. *Progeny not segregating*							
1. All progeny unbanded							
000	8						
003	1					1	
::3							
::y							
123							
2. All progeny banded unsegregating							
003	1						
::3	1	?+1	?1				
::y	18	?+2	?2				
123	4	?+1	?1	2		1	
B. *Progeny segregating*							
1. Unbanded and banded							
(a) According to unbanded progeny							
000	40			2		1	
003	3						
::3							
::y	4						
123			1				
(b) According to banded progeny							
000	39	?+2		3	?+2		
003	2	?+1			?+1		
::3							
::y	2	?+1			?+1		
123			1			1	
2. Banded							
(a) According to ::y							
003	3						
::3	3						
::y	3						
123						1	
(b) According to other banded forms							
003	4			1			
::3	3						
::y	4			1			
123						1	
Totals	143	8	6	8	4	6	175

TABLE 6. Range of variation of phenotype within the form 003.

Mating no.	Phenotype				
	00Y	003	:03 and 0:3	::3	
A. Only 003 in progeny					
TP 3A		40	14	5	59
B. 003 segregating from other forms in progeny					
TP 3B		24	13	1	38
TP 125		26	13	4	43
TP 139		6	2	5	13
TP 266		39			39
TP 267		21	4	1	26
TP 268		12			12
TP 269	1	25	1	1	28
TPR 231		21	1		22
Totals	1	214	48	17	280

seems no doubt that plain/dotty segregates independently from the banding/unbanding locus and that it is expressed in banded as well as unbanded forms. The expression in ::y at least (and perhaps other forms except 123) is little more disturbed, if at all, than in unbanded. The apparent agreements with Mendelian ratios but discrepancies with each other found between scores in different segregant classes requires further investigation.

003 and 00:

003 segregates clearly from 000, ::y and 123 (e.g. Figs. 1, 10, 11) and the segregations conform well to simple standard Mendelian ratios (Table 1). There can be some overlap with ::3, as shown in Table 6. In TP 3, the parents (Fig. 8) were an obvious 003, with only a trace of band 1, and an obvious ::3. Their progeny, TP 3A in Tables 1 and 6 were mainly 003, and indeed those scored in Table 6 as :03, 0:3 and ::3 almost all had weaker bands 1 and 2, or bands extending less far down the shell (see Fig. 9 for this variation as shown in the F_2), than did the ::3 parent. Table 6 exaggerates the overlap into ::3, which would be almost eliminated by a more elaborate scoring of the bands including their length and strength. A number of juveniles were left together in the breeding box to grow to a good size for scoring, and produced TP 3B (for which, therefore, the exact parents are not known) which can only be F_2 progeny. These give a good 3:1 segregation of 003 to ::3, a selection of which is shown in Fig. 9. There is no increase in apparent overlap from the F_1 generation, and the prolongation of the upper bands down the shell in ::3 enable it to be sorted easily from 003 with some additional banding (Fig. 9). In TP 125, 003 is recovered from a TP 19 offspring, with much the same range of variation as in TP 3A and B. In TP 266-8 the same lineage continues with 003 no more variable and occasionally apparently less so. The parents of TPR 231 come from a source isolated geographically (Majorca), but the progeny show no marked difference in variability.

In TP 139, 003 appears in a 2:1:1 segregation with more variation than usual towards ::3 (which does not appear in the progeny of this mating) as can be seen from the range shown in Fig. 1, the righthandmost shell in the 003 class being virtually ::3, as the parent 003 became on its later whorls. It is just possible that the parent is ::3, but in that case, one would not expect the range of variation seen in the progeny. In TP 279, there is an unusual spread of variation in ::3 towards 003. Several of the extreme individuals are shown in Fig. 6. The parents, however, and a large proportion of the offspring are ::3. In TPR 231 the form 00: varies somewhat in its additional bands, and the extremes are shown in Fig. 11. This lightly marked form, when with the upper bands, approaches the lightly marked parent

in TP 314 (Fig. 14, righthand parent) and might cause difficulty in scoring.

The strength of pigmentation of band 3 (and of the others) can vary considerably from black to a dusky yellow (Y) markedly stronger than that of the 00y modification of unbanded. In TP 269, a snail phenotypically 00Y crossed with a 003 gave much variation in the strength of band 3 in the 003 class, one offspring being like the parent 00Y but obviously as one extreme of a continuum. The parent 00Y, therefore, is probably also only an extreme of continuous variation, unless, as suggested previously, this very enigmatic individual is really ::y. In TP 264, a well-marked 003 from the progeny of TP 125 crossed with an apparently unbanded gives a 1:1 segregation of 000 and 00Y. It is possible that the 00Y are the heterozygotes for 003 and that in this case, the Y band is produced by superpenetrance.

With as few difficulties in scoring as this (only two really serious) it seems appropriate to rank 003 as a morph, but it may well be difficult to score against ::3 in some populations. Table 6 suggests that while very few shells indeed (a single 00Y) are liable to be mistaken for 00y, about 6% might be confused with ::3, although, as discussed above, there is usually a difference in the extent of development of bands 1 and 2.

The lightly marked form 00: in TPR 231 might either be an allele at the banding locus recessive to 000 and 003, or a modification of 003 mediated at a separate locus. From its association with apex colour in this mating, it behaves as an allele of 003 (see section on Apex Colour).

::3

This form is seen in Fig. 12 (mating TP 128) segregation from ::y and in Fig. 9 (TP 3B) from 003. Some of its variation towards 003 has been discussed under that heading; Table 7 suggests that about 18% of the progeny might be confusable with 003, but only about 3% really look like it. At the other end, about 5.5% may be very similar to 123. A single shell, a parent of TP 275, was scored at 8.1 mm as ::y, but rapidly developed the ::3 pattern. The progeny confirm that it was ::3 genetically, and this is a rare example of delay in the pigmentation of band 3 in this form. One parent of TP 128 was scored at 8.5 mm as 123 but was proved to be ::3.

::y

This is perhaps the most variable in expression of the forms, yet it segregates clearly from 003 and 123 (Fig. 10), from 000 and 003 (Fig. 1, yyy form of ::y) and without much difficulty from ::3 (Fig. 12). It happens that I have no segregation of it from 123 but the distinction should be very clear (compare the 123 parent with the ::y offspring in Fig. 10).

The principal variation is in the amount of dark pigment on bands 1 and/or 2, which may be considerable as in most of the ::y offspring in Fig. 10, varying to almost nothing as in the righthandmost ::y in Fig. 12, or without dark pigment as in the lefthandmost of the ::y shells in Fig. 1. This last variety, scored as yyy, might possibly be confused with some brown unbanded shells, but the yellow bands are separated by pale areas on light-coloured shells, as in Fig. 1 (contrast with the B000 shells in Fig. 11).

Table 8 gives the range of variation in my bred material. There is no suggestion that the individuals overlapping into 123 are more frequent in individuals heterozygous for 123 than they are in other individuals, but the numbers are too small for testing. Only about 2.5% overlap into ::: and 123, and only a

TABLE 7. Range of variation of phenotype within the form ::3.

Mating no.	Phenotype						
	003	:03, 0:3	Intermediate	::3	:::	123	Total
PB 12			16	51	5	1	73
TP 3B				10			10
TP 12		3	1	15			19
TP 15				7			7
TP 128		34	8	39	7		88
TP 279	7		28	15	1		51
	7	37	53	137	13	1	248

TABLE 8. Range of variation of phenotype within the form ::y.

Mating no.	Phenotype ::: & 123	::y	Inter-mediate	yyy	Other	Total
A. Both parents ::y, only ::y in progeny						
TP 31A		32	6	9		47
TP 31B		5	8	32		45
TP 236	3	103	3	21		130
TP 237	18	122	—	10		150
TP 238	2	66	13	11		92
TP 239		89	7	10		106
TP 240		55	5	6		66
TP 244		19	1	2		22
TP 274		29	15			44
B. Parents ::y and 123, only ::y in progeny; all progeny heterozygous 123						
TP 245		55	22	41		118
TP 246	1	69	1	2		73
TP 247	4	12	4			20
TP 250	3	13	2			18
C. ::y segregating in progeny						
PB 26		4	12	2		18
TP 29		7	1	15		23
TP 123		52	8			60
TP 128		50	3			53
TP 139		3	2	17		22
TP 241		28	3	4		35
TP 249		17	2			19
TP 251		13	4			17
TP 252	1	10	6	20		37
TP 253		3	2	15		20
TP 255		8	5			13
TP 257		14	3	4		21
TP 266		17	9	26		52
TP 267		19	3			22
TP 268		2	1		1*	4
TP 269		10	3	4		17
TP 275		27	15	12		54
TP 279	2	15		1		18
	34	968	169	264		1436

*Indicates a single 0::.

single shell (in 1436), phenotypically 0::, verges towards ::3. The proportion overall of yyy is about 18%. It varies in its preponderance, being specially abundant in TP 31B, TP 29, 252, 253, and 266. In TP 29 it seemed to segregate from ::y, but on very small numbers. There is no important heterogeneity in yyy overall, and only breeding experiments will determine whether it is a distinct form. In TP 31, two successive clutches from the same parents give significantly different scores (Table 8). It may not be genetically

determined, but phenotypically it seems to correspond to Cowie's yellow five-bandeds (this volume) which seem to have some genetic component.

Of the parents used, one in PB 26 was scored at 9.8 mm as ?::y as it had a faint indication of 2, but otherwise was a pale yyy. Genetically it was unbanded. One in TP 314 scored as yyy at 6.4 is certainly not a ::y (see Table 2 for its progeny) but may be a very lightly marked ::3. The others, 27 in all, were correctly scored. Although there is a slight error, this form may be called a morph.

123

This form, illustrated in Figs. 7, 10 (one parent) and 13, shows a full development of all 3 upper bands, although 1 and 2 may be fused and broken into blocks (Fig. 13) and band 3 is not usually as solid as in 003 and many ::3. The range of variation in my material is given in Table 9. About 7% overlap into ::y, and only a minute proportion into 00: and 000. These last were killed when still small. In the vast majority the banding starts very early, almost as soon as a new whorl is added to the embryonic shell. However, the more bands on the shell, the more variation can be displayed. Cowie (this volume, Fig. 8) shows some marked interpopulation variation in 123. Table 9 suggests that there are differences in range in different broods. Of the 10 parents used, 4 are certainly rightly scored, and there is no evidence that the remainder are not. When this form has been investigated more, it is probable that more overlaps and confusions will be found. It is often abundant in the wild, like the 5 banded form of *Cepaea nemoralis* and may provisionally be ranked as a morph.

hz and shz

In the dead-white of the shell and the colourless apex, hz and shz stand out from all other forms, but can be confused with each other if banding is not well developed, which happens in some small shells that have not yet begun to develop it, and of course in totally unbandeds.

Apex colour

In TP 236 (Table 10) all but a very few of the progeny from two dark-apexed parents are dark, and inspection of the shells shows that all the progeny form a single continuum in respect of apex colour. Almost all the dark apices are only partly dark, some with only a slight touch of dark pigment, 2 (dubious) with only a faint flush of brown, and 3 with none at all. A similar variation is seen in other matings, and where only a few anomalous apices are found, they can be regarded as extremes of the abundant class. (A few shells are marked as dubious because they are somewhat worn and the apex colour is obscured.) If extremes are counted in with the class to which they probably belong genetically and dubious scores are ignored, then the matings shown in Table 10 are consistent with pale being dominant to dark apex and closely linked to the unbanded/banded locus. In

TABLE 9. Range of variation of phenotype within the form 123. The variation in TP 203 is not disjunct but is made to appear so by the particular symbolization.

Mating no.	Phenotype								
	::y	::Y	123 → ::Y	123	:::	::3	00:	000	Total
TP 104		11	19	18					48
TP 201			2	20					22
TP 202		3	3	7	23				36
TP 203	1	7	7		49		1		65
TP 206			19	2	14				35
TP 207			8	16	9	3			36
TP 208				3	21				24
TP 209	12		22		7				41
TP 211	5		2	11	3				21
TP 282					234		2	1	237
	18	21	82	77	360	3	3	1	565

TABLE 10. Apex colour.

Mating no.	Parental scores	Classes of progeny	Apex score in progeny			
			Pale	Dubious	Dark	Total
PB 12	000 pale	000	155	31	12	198
	000 pale	::3	8	10	65	83
TP 128	::3 dark	::3			45	45
	::y dark	::y			53	53
TP 236	::y dark	::y	3	2	167	172
	::y dark					
TP 241	::y dark	000	30			30
	(hz)	::y	1		33	34
TP 244	::y dark	::y			22	22
	::y dark					
TP 252	000 pale	000	44			44
	(hz)	::y			37	37
TP 267	003 dark	003			26	26
	123 dark	::y	1		21	22
TPR 231	B 000 dark	B 000	51	1	51	103
	003 pale	003	1	7	52	60
		00:	46	3		49
			340	54	584	978

TPR 231 (Fig. 11) there is a 1:1 ratio of pale to dark apex within the brown unbandeds. Within the bandeds there is a clear association in 1:1 ratio of pale with 003 and dark with 00:. This is consistent with one parent being brown unbanded homozygous dark, and the other 003 heterozygous pale. The occurrence in quantity of both dark and pale apex within the brown unbandeds shows that it is not a question of genes producing dark pigment on the shell also colouring the apex. Cowie's material also shows this.

The table shows that, for example, only 14 out of 340 are wrongly scored as pale; even if we assign the dubious shells alternately to pale and dark, the erroneous scores would increase only to 28, i.e. about 4% and 8% respectively, with similar estimates for the dark ones. The progeny agree well with all the scorable parents (14), hyalozonates having, of course, a colourless apex. The hyalozonates are carrying alleles for apex colour which is therefore at a separate locus from hz, and hypostatic to it.

DISCUSSION

It is clear from the work reported above, and from Cowie's (1982, and this volume), that much of the variation in shell pattern in *Theba pisana* so far investigated is mediated by major genes, much as in *Cepaea nemoralis* (e.g. Cain *et al.*, 1968). If the brown shells in TPR 231 owe their colour to a general shell pigment, not to a spread of banding pigment, as seems likely from the whole shell being coloured right to the umbilicus, brown shell colour is linked to the unbanded/banded locus. Apex colour is certainly at a distinct locus closely linked to unbanded/banded. These are the only two examples of linkage found so far. Lip colour, and dark/yellow banding (Cowie, this volume) are also at separate loci, as is the plain/dotty segregation. Hyalozonate and subhyalozonate, as against normal pigmentation are also separate.

The following symbols are proposed:—

B absence of bands and presence in various patterns: B^0 unbanded, dominant to bands present, with alleles: B^{003}, $B^{::3}$, $B^{::y}$ and B^{123} in that order of descending dominance. $B^{00:}$ should be inserted here after B^{003} but exactly where is not yet known.

A apex colour: A^P pale apex, dominant to A^D, dark apex. *P* pigmentation of bands: P^N normally pigmented bands, dominant to P^T, transparent bands, or hyalozonate. (If subhyalozonate is an allele at this locus, it could

be designated as P^{ST}; the relationship between P^{ST} and P^{T} is not yet known.) *M* (for *maculata*), plain/dotty: M^{P}, plain, dominant to M^{D}, dotty. As this variation in *Theba pisana* seems completely different from the punctate form in *Cepaea nemoralis* (locus *I*), which is a modification of all the bands present and not expressed at all in unbandeds, a different symbol is necessary. Other forms are best referred to by formula until they are better known.

While the 123 form (usually 12345) in *T. pisana* corresponds to the recessive, and abundant, 12345 in *C. nemoralis,* the other principal banding forms in *T. pisana* differ considerably. They are not mediated by unlinked modifiers, like 00300 and 00345 in *C. nemoralis,* and though they may be mediated by a series of loci extremely tightly linked to an unbanded/banded locus, as are punctate bands, spread bands and albolabiate in *C. nemoralis,* their modes of variation do not correspond at all to these forms (except perhaps lip colour). Even the morph 003 which corresponds closely to 00345 in *C. nemoralis* in banding formula differs in other respects. In the whiteness of the area above band 3, not specially differentiated in any *Cepaea,* it corresponds far better with a form, of the same phenotype and often scorable as an independent morph in random samples, in various species of helicelline snails (Cain, in prep.). The yellow bands have no parallel in *Cepaea.* In one respect, there is a similarity; in *C. nemoralis,* except for hyalozonate and albolabiate, the fewer the bands and the less the pigment, the more dominant or epistatic the form. The same is true for *Theba pisana,* including apex colour, but with the exception of ::y (which, as shown above, includes yyy).

The variation in *Theba pisana* differs from that in *Cepaea nemoralis* not only in the patterns produced and in the genetic control of banding forms, but also in the precision with which forms are inherited. It is suggested that unbanded and 003 are definite enough to be called morphs, and that probably ::3, ::y and 123 might be admitted into this category. But while the morphs in *C. nemoralis* are almost invariably clearcut (some occasional exceptions are mentioned by Cain, 1977), an overlap of a few percent at each end of the range of each morph in *T. pisana* occurs regularly, and in ::y a greater range of variation is found within the morph than is seen in any in *C. nemoralis.* (The nature of the variation in 000 and 00y is too uncertain to allow its basis to be determined.) The effect of this interconnection of forms is to make it difficult to score random samples from the wild, as many previous authors have found (Cowie, this volume).

Although the evidence at present is not enough for any firm conclusion, there are indications in the tables given here that progeny of similar matings, and even of replicates can differ noticeably in the range of variation shown within the same form; moreover plain and dotty may differ in their ratio even between segregant 000 and banded in the same brood. It is difficult to see how this can be brought about in the breeding boxes except by polygenic modifiers, some at least linked to the *B* locus. In some replicate matings recently scored, there appear to be segregations in some progeny and continuities in others with different preponderances of particular variants (Cain, in prep.). This again suggests considerable interference by the genetic background.

Some authors think it quite useless to ask what the significance of such types of variation as that described here might be. Gould and Lewontin (1979), for example, exhort us to abandon what they describe as the adaptationist programme and contemplate the whole animal, but they give no procedure for doing this. It may still be worthwhile to ask adaptationist questions, because if we do not, we shall certainly not find answers, even when those answers are there to be found. The whole of the work in *Cepaea* (e.g. Cain, 1983; Jones *et al.,* 1977) is a case in point, carefully ignored by proponents of the whole-animal approach, as Clarke (1978) and Cain (1983) have pointed out. In other helicine snails (Cain, in prep.) what variation has been investigated seems to be like that in *Cepaea,* fairly clear-cut. My experience in scoring shell colour and pattern variations in British winkles, however, suggests a situation very like that in *T. pisana.* No difference between *Cepaea* on one hand, and *T. pisana* and winkles on the other is apparent in small samples, in which, since there are few individuals in each category, distinctions are easy. With increasing size of sample, more and more intermediates are found in *T. pisana* and in winkles (*Littorina mariae, rudis* etc.), but not in *C. nemoralis,* in which the morphs stay well-defined. As snails are extremely localized during their life-history, this means that a predator searching any small area will have difficulty in recognising most of the

forms if it gets a hunting image based on any one of them. It is possible that the mode of variation found in winkles and *T. pisana* means that many major variants are so unstandardized that it is less easy to form hunting images except for a small part of each. For example, a bird finding yyy in *T. pisana* and thereafter looking for it might tend to overlook even ::y which is part of the same morph, or one finding 003 might tend to miss 0:3 and :03. Certainly selection, of whatever sort, against the phenotype 003 (for example) will be less effective if some individuals carrying that allele are disguised as ::3, 00:, or 00Y.

But if this is the explanation, one may well ask why the same system of variation is not found in *C. nemoralis.* Remarkably, the answer may be that it is, in spite of what has just been said. Population geneticists naturally work with variants that can be scored unambiguously and have known genetic bases, and these are what have been elucidated genetically so far in *C. nemoralis.* There is a vast amount of apparently polygenic variation in the five-banded form, with weakening or strengthening of banding pigment, reduction to actual suppression of any band, and broadening of any until they fuse with their neighbours. Counting only fusion and absence of bands, there are 89 possible variants (Taylor, 1910) in all of which the degree of pigmentation, time of appearance and extent along the whorls, and width may vary considerably. Moreover, the numerous alleles known to mediate shades of colour within the three main colour classes (Cain *et al.,* 1968, give 6 with a probable 3 others) do produce a considerable variety of shades, modified by the presence or absence of banding. The repertoire of variation in *Cepaea nemoralis* is of course as appropriate to its range of backgrounds as is that of *T. pisana.* The full variation in *Cepaea* has hardly ever been scored in any published paper that I know of. Only in climatically rather extreme conditions is the range of variation restricted to a few well-marked morphs (see Jones *et al.,* 1977 for a review). It is possible that the variation in *Cepaea* also is such as to make the production of a hunting image comprising many of the individual prey in a small area difficult. This suggestion is open to experimental investigation.

The genetic means used by *T. pisana* to obtain a blurring of the morphs needs further investigation, but certainly seems to include both variable penetrance and segregation modifiers. There appears to be no good natural history of penetrance. On asking well-known geneticists about it, I have been told that it is a very old-fashioned subject which caused some perturbation among early students of Mendelizing characters. I doubt whether snails would think of it like that.

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LITERATURE CITED

CAIN, A. J., 1977, The efficacy of natural selection in wild populations. In: GOULDEN, C. E., ed., *The changing scenes in natural sciences, 1776–1976,* Academy of Natural Sciences, Philadelphia, p. 111–133.

CAIN, A. J., 1983, Ecology and ecogenetics of terrestrial molluscan populations. In: WILBUR, K. M., ed.-in-chief, and RUSSELL-HUNTER, W. D., ed., *The Mollusca,* vol. 6: *Ecology.* Academic Press, p. 597–647.

CAIN, A. J., SHEPPARD, P. M. & KING, J. M. B., 1968, Studies on *Cepaea* I. The genetics of some morphs and varieties of *Cepaea nemoralis* (L.). *Philosophical Transactions of the Royal Society of London,* ser. B, 253: 383–396.

CLARKE, B. [C.], 1978, Some contributions of snails to the development of ecological genetics. In: BRUSSARD, P. F., ed., *Ecological genetics: the interface.* Springer-Verlag, New York, p. 159–170.

COWIE, R. H., 1980, Precocious breeding of *Theba pisana* (Müller) (Pulmonata: Helicidae). *Journal of Conchology,* 30: 238.

COWIE, R. H., 1982, Studies on the ecology and ecogenetics of the helicid land snail *Theba pisana* (Müller). Ph.D. thesis, University of Liverpool.

COWIE, R. H., 1984, Ecogenetics of *Theba pisana* (Pulmonata: Helicidae) at the northern edge of its range. *Malacologia,* 25: 361–380.

GOULD, S. J. & LEWONTIN, R. C., 1979, The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. In: SMITH, J. M. & HOLLIDAY, R., ed., *The evolution of adaptation by natural selection,* The Royal Society, London, p. 581–598.

HELLER, J., 1981, Visual versus climatic selection of shell banding in the landsnail *Theba pisana* in Israel. *Journal of Zoology, London,* 194: 85–101.

HUXLEY, Sir J. S., 1955, Morphism and evolution. *Heredity,* 9: 1–52.

JONES, J. S., LEITH, B. H. & RAWLINGS, P., 1977, Polymorphism in *Cepaea:* a problem with too many solutions? *Annual Review of Ecology and Systematics,* 8: 109–143.

MURRAY, J., 1975, The genetics of the Mollusca. In: KING, R. C., ed., *Handbook of genetics,* Plenum, New York, p. 3–31.

SACCHI, C., 1952, Ricerche sulla variabilità geografica in popolazioni Italiane di *Euparypha pisana* Müll. (Stylommatophora Helicidae). *Annali del Museo civico di Storia naturale di Genova,* 65: 211–258.

SACCHI, C., 1955, Fattori ecologici e storici nel polimorfismo delle "Euparypha" ("Helicidae Helicinae") del Marocco occidentale. *Studia Ghisleriana,* ser. 3, 2: 44–66, 1 pl.

TAYLOR, J. W., 1895, *Monograph of the land and freshwater Mollusca of the British Isles.* Taylor Brothers, Leeds, Part 2, pl. 2.

TAYLOR, J. W., 1910, *Monograph of the land and freshwater Mollusca of the British Isles.* Taylor Brothers, Leeds, Part 17, p. 287–294.

TAYLOR, J. W. 1912, *Monograph of the land and freshwater Mollusca of the British Isles.* Taylor Brothers, Leeds, Part 19, pl. 30, 31.

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CYTOGENETIC STUDY OF INTERSPECIFIC HYBRIDS OF *ASHMUNELLA* (MOLLUSCA: PULMONATA: POLYGYRIDAE), I. *A. PROXIMA* × *A. LENTICULA* F_1 HYBRIDS

Noorullah Babrakzai[1] & Walter B. Miller[2]

ABSTRACT

Twenty interspecific hybrids of *Ashmunella proxima albicauda* Pilsbry & Ferriss × *A. lenticula* Gregg obtained from a cross of virgin snails of the parental species were reared in terraria under laboratory conditions. After reaching maturity, the ovotestes of the hybrids, which are intermediate in shell morphology, were examined cytologically. Karyotypes of the parental species were constructed from developing embryos of the parental species populations. *A. proxima albicauda* has four distinct chromosomes with large C-band positive heterochromatic long arms in the mitotic metaphase. Such chromosome markers are absent in the *A. lenticula* karyotype.The hybrids have half of the *A. proxima* chromosome markers in both meiosis and mitosis. The meiotic behavior of chromosomes in hybrids ranged from complete synapsis to signs of asynapsis in pachytene. Univalent and multivalent formation, evidence of translocations and stickiness of meiotic chromosomes, were observed in all stages of the meiosis. We summarize our conclusions as follows: 1) *A. proxima* and *A. lenticula* are two closely related but cytologically and genetically different species; 2) the karyotypes of the two species have evolved differently; 3) the observed cytological differences in karyotypes of the two species may represent a gross oversimplification of their genetic differences; 4) the incidence of observed normal synapsis in the hybrids may be due to a favorable expression of *pairing genes* of the two genomes suppressing multivalent and univalent formation in meiosis or lack of chiasmata due to chance, or both; 5) conservatism in synonymizing land snail species of the arid Southwest is strongly recommended.

Key words: cytology; cytogenetics; interspecies hybrids; meiosis; C-bands; marker chromosomes; chromosomal aberrations.

INTRODUCTION

The desert southwest of the United States and the northwest of Mexico provides a vast area full of interesting and challenging problems to students of evolutionary biology. Some of the most perplexing problems occur in terrestrial systematic malacology.

The hot, arid valleys constitute a geographical isolating barrier to the land snail populations of the mesic, forested mountains equally as formidable as oceans to island populations. The geographical barriers vary in degree, depending on elevation, and some populations of snails, now isolated, may have been connected in relatively recent pluvial periods, some eight to ten thousand years ago.

Ernst Mayr's definition of a biological species, based on reproductive isolation of natural populations, has been used by many terrestrial malacologists as the fundamental criterion in distinguishing species among land snail populations. Unfortunately, there is no simple, direct method to determine reproductive isolation, and the systematist must resort to inferring such isolation by estimating genetic differences sufficient to cause incompatibility in such critical areas as behavior, copulation, gametogenesis, etc.

In systematic malacology, the most obvious indicators of genetic differences have been the morphology of the shell and the anatomy of the reproductive system. In certain genera, however, closely related species exhibit only minor differences in morphology and essentially no consistent differences in anatomy. One such genus is *Ashmunella* Pilsbry & Cockerell, 1899. In the Chiricahua Mountains of Arizona, malacologists have recognized a large number of species (Pilsbry, 1940), many of which appeared to differ by only minor characteristics. Bequaert & Miller (1973) reviewed the classification of this

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group and concluded, intuitively, that certain species should be synonymized or relegated to subspecific status (i.e. genetic differences insufficient to cause reproductive isolation). Subsequently, however, one of us (WBM) encouraged graduate students to investigate genetic differences at the cytological level. Regrettably, it was found that classical cytological studies were not particularly elucidating. For example, the haploid chromosome numbers of *A. proxima albicauda* Pilsbry & Ferriss, 1910, and *A. lenticula* Gregg, 1953, are n = 29 (Reeder, 1975). Fifteen additional species of *Ashmunella* have the same haploid and diploid chromosome numbers (Reeder, 1975; Reeder *et al.,* 1975; Reeder & Miller, 1974; Babrakzai, in press; and Babrakzai, unpublished). Similarly, electrophoretic studies utilizing foot muscle proteins have been inconclusive on species of *Ashmunella* from the Chiricahua Mountains (Trifan, 1976). In light of the foregoing, we thought it worthwhile to hybridize *A. proxima albicauda* with *A. lenticula* in the laboratory and to obtain F_1 hybrids and to study the following:

(i) Expression of the two genomes in the hybrid phenotype.

(ii) Meiotic pairing configurations that might indicate the degree of homology of the two genomes and reveal any changes in the structural arrangement of the chromosomes in each species.

(iii) Confirmation of the hybrid nature of the F_1 offspring by tracing specific marker chromosomes of the parental species in mitosis and meiosis.

In this communication, we report the results of a study of the chromosomes of *A. proxima albicauda* and *A. lenticula* and their F_1 hybrids utilizing standard and centromeric banding techniques.

MATERIALS AND METHODS

A. *Collection of Snails*

Specimens were obtained from the following localities:

1. *Ashmunella lenticula* Gregg. Horseshoe Canyon at the mouth of Pothole Canyon, Chiricahua Mountains, Cochise County, Arizona; 35 adult (collected in 1976) and four immature specimens (collected in 1971).

2. *Ashmunella proxima albicauda* Pilsbry & Ferriss. Jhus Canyon, Chiricahua Mountains, Cochise County, Arizona; 14 adults (collected in 1976) and four immature specimens (collected in 1971).

B. *Maintenance and Breeding*

The ovotestes of pulmonates have been used to obtain chromosome squash preparations in the past (Babrakzai & Miller, 1974). However, for mitotic chromosome studies involving banding techniques, the use of ovotestes has proved to be wasteful and results in sacrificing precious specimens. Therefore, a breeding program was devised to obtain mitotic chromosomes from the developing embryos of parental snails. The snails were kept in redwood terraria with leaf litter and some rocks from their native habitats. Constant temperature (30°C) and humidity (50%) was maintained and they were fed romaine lettuce once a week. The parental colonies were allowed to estivate during the normal dry periods at their native localities and were activated at the onset of the rainy seasons by keeping the terrarium moist and feeding lettuce to the snails.

At the time of feeding, all the fecal material from the walls of the terraria, roofs, etc., was removed with moist paper towels. The snails were kept clean by gently washing their shells, in order to reduce the possibility of bacterial or fungal infections. In approximately 6–8 weeks, they started to breed, laying eggs at irregular but frequent intervals, so that once or twice a week, after feeding, we were able to collect one or more clutches of eggs from a terrarium. Approximately 300 eggs were dissected and processed for cytological study.

Most snails bury their eggs below the surface of the mulch by digging into the mulch with their heads, aided by the muscular action of the foot. It was therefore necessary to gently scrape the entire layer of mulch at the bottom of the terrarium with a small spatula. The eggs were removed from the terrarium, placed in a petri dish, and washed in tap water to remove all the debris and most of the small nematode worms that feed on the albumin if the egg capsule is accidentally ruptured. The eggs were then covered in moist paper towels and labelled with the date of collection. The embryos from these eggs were used for mitotic chromosome study.

C. *Hybridization*

Pulmonate land snails are hermaphroditic. Adult specimens collected from the field are unsuitable for hybridization experiments because of the possible presence of exogenous sperm in their spermathecae. Accordingly, virgin (sexually immature) specimens of *A. proxima albicauda* and *A. lenticula* were placed together in a terrarium (one specimen from each species). The maintenance procedure was as described earlier.

Only one pair of virgin parental snails reached maturity in 1975 and copulation between them was observed in the same year; subsequently, they laid eggs. The newly hatched, presumed hybrid snails were reared with special care and attention. Old lettuce leaves from the terrarium had to be examined carefully to make sure that the very small snails were not lost accidentally. The first set of F_1 offspring reached sexual maturity in March, 1980, when reflected lips of their shells were observed, indicating a halt to shell growth. Twenty presumed F_1 hybrids were subsequently used for cytogenetic studies in the laboratory.

D. *Preparation of Mitotic Metaphase Spreads From Embryos*

The petri dishes containing eggs were incubated at room temperature inside terraria, with water-filled finger bowls to maintain high humidity and reduce the chance of egg desiccation.

The incubation period varied from 5 to 8 days. After incubation, the eggs were placed in a watch glass in 0.4% sodium citrate solution and dissected individually under a binocular microscope to release the embryos into the citrate solution. The embryos were then washed in fresh citrate to remove the albumin, incubated for 30 minutes to one hour in the citrate hypotonic solution, and then treated with enough 10-3 M colchicine to make an approximate final concentration of 10-4 M solution. This was followed by further incubation for one hour.

The embryos were fixed in methanol acetic acid (3:1) for one hour and then placed in 45% acetic acid for 10–15 minutes. One embryo, in a small drop of 45% acetic acid, was placed on a clean microscope slide, macerated with a very fine needle and air dried, followed by a 10–30 sec. treatment in absolute ethyl alcohol, and air dried again.

The slides were then placed in clean slide storage boxes to allow the chromosomes to "age." Such "aging" seems to be important for the induction of C-bands. Seven-to-ten-day-old slides yielded best results. The unstained slides were scanned under a microscope and slides with fewer than five good chromosomes spreads were discarded. For routine chromosome work, the slides were stained in 2% Harleco Giemsa for 2-3 minutes at pH 6.8, rinsed in distilled water, and air dried. Permanent mounts were made using Euparal.

E. *The C-Banding Technique*

The "aged" slides were treated according to the BSG-Technique of Sumner (1972), with the following modifications:

1. Placed in 0.2 N HCl at room temperature for one hour.
2. Treated in 5% barium hydroxide at 60°C for 5 minutes.
3. Rinsed in distilled water and placed in 0.2 N HCl.
4. Rinsed in distilled water.
5. Incubated in 2X SSC (0.3M NaCl and 0.03M sodium citrate) at 60°C for one hour.
6. Rinsed in distilled water.
7. Stained in 2% buffered Harleco Giemsa pH 6.8 for 5-30 minutes.
8. Rinsed in distilled water and air dried.
9. Mounted in Euparal.

The slides were scanned and the coordinates of the chromosome spreads were recorded using a "Micro Locator" (retailed by Van Waters & Rogers). Photomicrographs were taken using Kodak high contrast copy and Kodak Technical pan films at ASA ratings of 6–24, using a Wild microscope and Nikon PFM system. To enhance contrast, a yellow filter, or a green filter or their combination, was used. Prints were made with Kodak Polycontrast Rapid RC paper using Kodak Polycontrast Filters.

F. *Squash Preparations*

The ovotestes of the F_1 hybrids were used for cytological studies. Chromosome squash preparations were made using the hypotonic squash technique (Babrakzai & Miller, 1974). No colchicine treatment was applied to the ovotestes. The fixed tissues were squashed

in 45% acetic acid on microscope slides. The slides were frozen in dry ice for 2–4 minutes and the coverslips were removed using a sharp razor blade. The slides were dipped in methanol and divided into three batches as follows:

a. The first batch was stained with orcein or Giemsa and permanent mounts were made with Euparal for the study of mitotic and meiotic chromosomes.

b. The second batch was processed using the C-banding technique (followed by staining in Giemsa). This batch was prescanned for mitotic metaphases.

c. The third batch was stained with Giemsa and made permanent as in (a) above. The meiotic chromosome spreads from 100 cells were studied and recorded on photographic film. Then the coverslips were removed, the slides were washed in three changes of absolute ethanol, destained in 70% alcohol and 0.2 N HCl. The C-banding technique was applied to these slides as described above with the following modifications:

1. Treatment in barium hydroxide was changed from 30 sec. to 3 min.

2. The temperature of the barium hydroxide solution was found to be another important variable for the squash preparations from the ovotestes of these snails. Therefore, we lowered the temperature of the barium hydroxide to 50°C and no water bath was used. Treatment temperature varied between 40°C to 50°C. Permanent mounts were made after 2 × SSC treatment for one hour and staining in Giemsa. The meiotic chromosome spreads were retraced and good preparations were photographed under the microscope for comparison with the same spreads before the application of the C-banding technique.

RESULTS AND DISCUSSION

Specimens of *Ashmunella proxima albicauda* and *A. lenticula* can be identified on the basis of shell morphology. In *A. p. albicauda,* the outer lip of the shell has a single tooth with 2 to 3 inconspicuous indentations, two fused basal lip teeth, the outer one being more prominent, and a single parietal tooth which is more or less oblique (Fig. 1). The shell of *A. lenticula* on the other hand, has a relatively shorter outer lip tooth with a smooth margin, and two basal lip teeth that are separate from each other; the parietal lip tooth resembles that of *A. p. albicauda* (Fig. 2). The hybrid shell is intermediate in morphology (Fig. 3), i.e. the outer lip tooth is shorter than in *A. p. albicauda* and slightly bigger than in *A. lenticula.* The basal lip teeth are farther apart than those of *A. p. albicauda* and less so than in *A. lenticula.* This intermediate nature of the hybrid shell was important in the initial diagnosis as a first indication of the success of the experiment after nine years! However, this observation by itself is not a conclusive proof of their hybrid nature and did not answer the question we raised earlier. Since polygyrid snails are functional hermaphrodites, and self-fertilization has been reported in *Triodopsis* (McKracken & Brussard, 1980), it was theoretically possible that the offspring could have resulted from one of the following processes:

(i) self-fertilization
(ii) parthenogenesis
(iii) sperm activation without amphimixis
(iv) cross-fertilization

After C-banding treatment, the *A. p. albicauda* karyotype showed two large pairs of homologous chromosomes with two-thirds of the long arm being C+ (i.e. heterochromatic) in each case (Fig. 4). Thus, they stand out easily in C+ preparations. We will refer to these marker chromosomes as 4 *m*–q C+ and 5 *m*–q C+, since they are the 4th and 5th largest chromosomes with median centromere (m), (q stands for the long arm of a chromosome and C+ for the interstitial band of heterochromatin). A complete discussion of the karyotypes in the light of C-bands and G-bands (Giemsa bands) of the *Ashmunella proxima* species group will be communicated in the future. When the C-banding pattern of *A. p. albicauda* is compared with *A. lenticula* (Fig. 5), it becomes evident that the C+ bands seen in 4 *m*–q C+ and 5 *m*–q C+ of *A. p. albicauda* are lacking in *A. lenticula.* The C-banding pattern of mitotic chromosomes from the hybrids (Fig. 6) revealed only one-half, i.e. two of the four marker chromosomes of *A. p. albicauda.* We were, therefore, convinced that cross-fertilization between *A. p. albicauda* and *A. lenticula* did indeed occur.

The C-banding pattern of the karyotype of a species provides new insights into the cytology of related taxa due to the ease with which specific marker chromosomes can be traced in hybrids. For example, C-banding pattern has been useful in the demonstration of the two parental genomes (horse and donkey) in

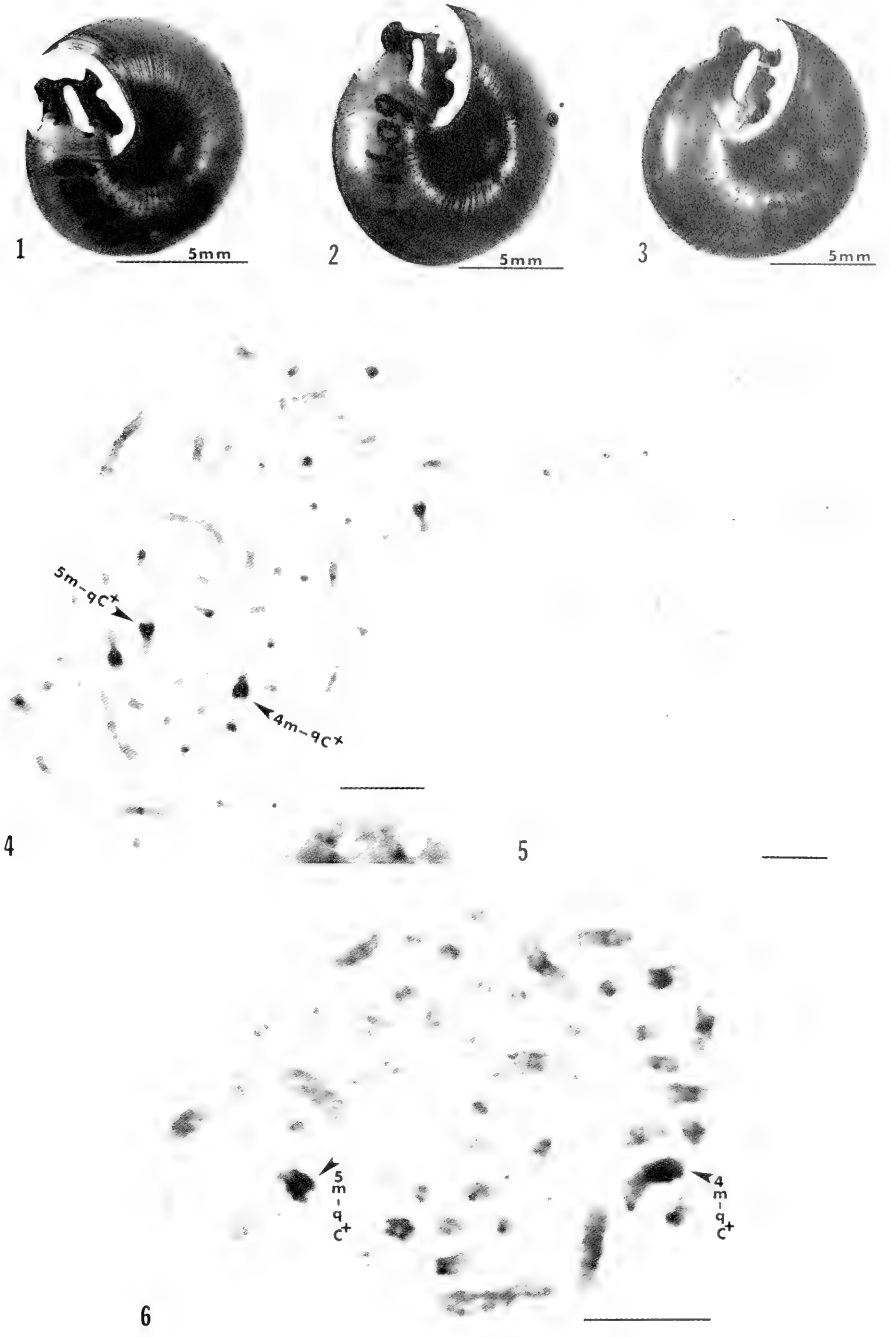

FIG. 1. Shell of *Ashmunella proxima albicauda* (parent). FIG. 2. Shell of *A. lenticula* (parent). FIG. 3. Shell of *A. proxima albicauda* × *A. lenticula* F_1 hybrid. FIG. 4. C-banding pattern of mitotic metaphase chromosomes of *A. proxima albicauda* with the marker chromosomes; 4 *m*–q C + and 5 *m*–q C + (the bar in Figs. 4–25 is 10 μm). FIG. 5. C-banding pattern of mitotic metaphase chromosomes of *A. lenticula*. FIG. 6. C-banding pattern of mitotic metaphase chromosomes of *A. p. albicauda* × *A. lenticula* F_1 hybrid, with one-half of the *A. p. albicauda* markers.

the mule (Cribiu & De Giovanni, 1978), in wheat and rye hybrids (Shchapova & Zaripova, 1979), in *Triticale* × *Secale cereale* hybrids (Jouve *et al.,* 1980), in interspecies hybrids of the red muntjack deer *Muntiacus muntjack vaginalis* and the Chinese muntjack *M. reevesi* (Liming *et al.,* 1980), and in hybrid newts, *Triturus cristatus carnifex* crossed with *T. marmoratus* (Mancino *et al.,* 1979). The C-banding pattern of the karyotype has also revealed a new phenomenon of chromosomal polymorphism in some species. For example, chromosome nos. 1, 3, 7, 8, 10, 11 and 13 in the black rat, *Rattus rattus tanezumi,* are polymorphic in regard to the C-bands and show a regular segregation in the offspring with Mendelian ratio (Yosida, 1979). A similar polymorphism has been observed in chromosome no. 15 in domestic pigs with Mendelian mode of transmission (Christensen & Seedegard, 1979). The pulmonate land snails are apparently no exception to such cytological phenomena. We have observed C-band polymorphism in two chromosomes of *Ashmunella rhyssa altissima* from the Sierra Blanca-Sacramento Mountains of New Mexico (Babrakzai, in press).

In the absence of critical cytogenetic data, however, the intermediate nature of the F_1 hybrid snail shells and the presence of one-half markers of each parental species do not necessarily confirm or reject reproductive isolation. The study of meiosis in the F_1 hybrids did, however, yield some meaningful results on the speciation problem (Table 1). In the parents, normal synapsis in pachytene (Fig. 7) of *A. p. albicauda* and late diakinesis in *A. lenticula* (Fig. 8), with 29 bivalents, and no apparent indications of chromosomal aberrations (i.e. translocation and inversion heterozygosities, univalents, etc.), can easily be followed.

The pachytene stage in the hybrids, however, revealed the occurrence of univalents (Fig. 9), and complex multivalents (Figs. 10, 11). It was apparent that certain chromosomes of each parental genome were not genetically identical perhaps due to exchanges and/or mutations, resulting in asynapsis. Chromosomal associations in diplotene, diakinesis and metaphase I range from almost complete synapsis with one odd bivalent (Fig. 12) to signs of asynapsis. The odd bivalent in question (Fig. 12) consists of one marker chromosome, probably 4 *m*–q C+ or 5 *m*–q C+ of *A. p. albicauda,* partially synapsing with its counterpart from *A. lenticula.* The heterochromatic end of the *A. p. albicauda* chromosome has failed to make a chiasma and, therefore, the bivalent has the shape of a chain of 2 chromosomes. Such bivalents were not encountered in the parental meiosis. However, the rest of the bivalents appear "normal." Table 1 summarizes the results of the study on both meiotic and mitotic chromosomes.

A meiotic chromosome spread was defined as "normal" if one could count 29 bivalents regardless of their shapes. On the other hand, if univalents, multivalents, stickiness of chromosomes, etc., were encountered in a cell undergoing meiosis, then it was defined as "abnormal." These rather arbitrary and descriptive terms were useful in recording the data. A more accurate and precise study and its interpretation would have been beyond our means. Further study of meiotic spreads revealed chromosomal aberrations of various kinds in the hybrids resulting in many univalent and multivalent associations. The largest chromosome pair in meiosis is easily recognizable and appears morphologically identical in both species. Tracing the bivalent produced by this chromosome pair led to

TABLE 1. Analysis of meiotic and mitotic chromosomes of *Ashmunella proxima albicauda*, *A. lenticula*, and their F_1 hybrids. Legend: N = Normal synapsis; Abn = Abnormal synapsis; C+ = C-banded; C− = no C-banding treatment.

	Pachytene		Diplotene		Diakinesis		Metaphase I		Metaphase II		Mitosis		
Organism	N	Abn	N	Abn	N	Abn	N	Abn	N	Abn	C+	C−	Total
A. proxima albicauda	47	—	23	—	25	—	—	—	—	—	21	23	139
A. lenticula	10	—	4	—	18	—	1	—	—	—	20	20	73
Hybrids	15	64	—	51	17	62	—	19	6	21	47	53	355
Total	72	64	27	51	60	62	1	19	6	21	88	96	567

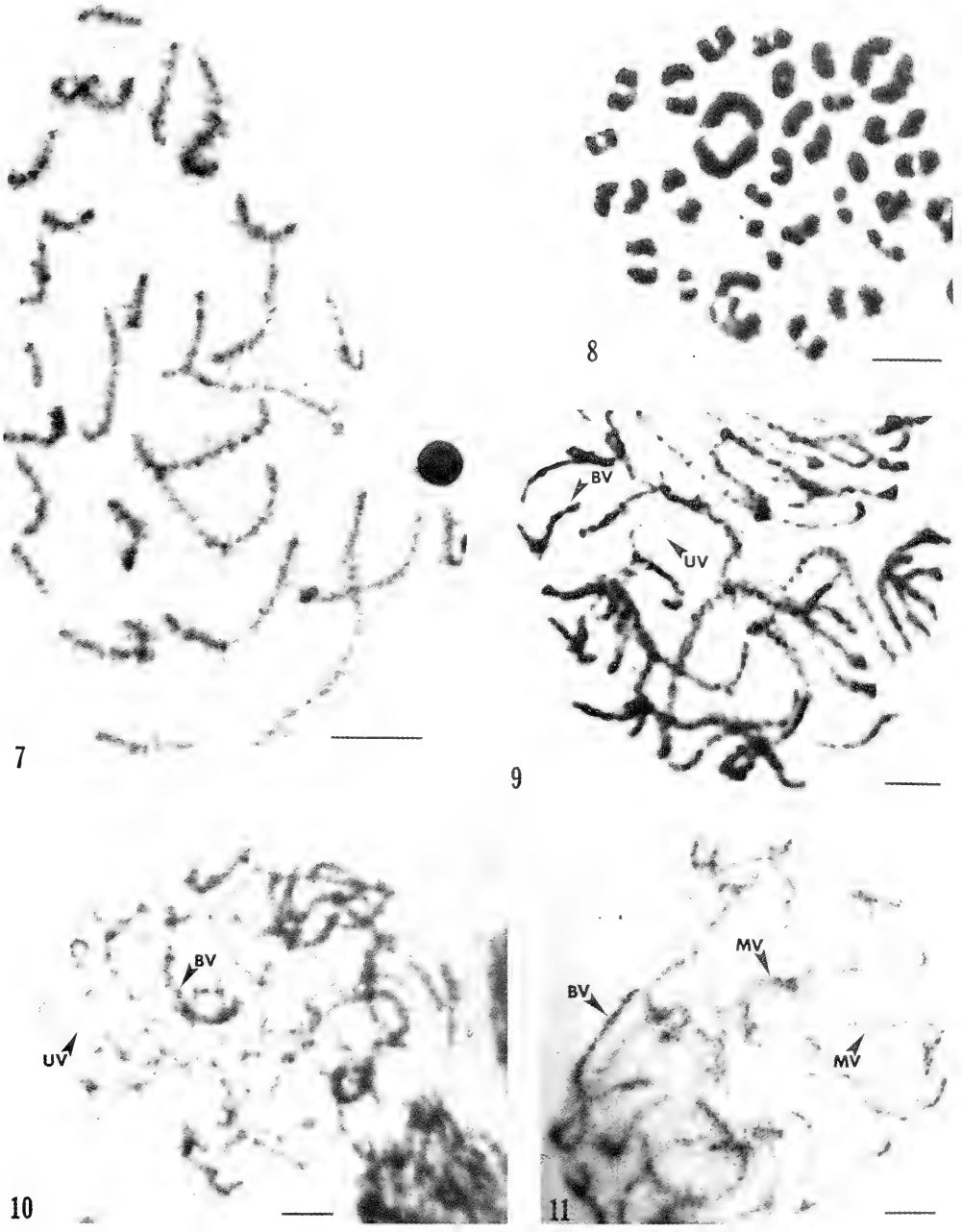

FIG. 7. Composite picture of the pachytene stage chromosomes of *A. p. albicauda* with normal (control) synapsis. FIG. 8. Diakinesis chromosomes of *A. lenticula* with normal (control) bivalents. FIG. 9. Pachytene stage of meiosis in the *A. p. albicauda* × *A. lenticula* hybrid, UV = univalent, BV = bivalent (for comparison). FIG. 10. Pachytene stage in the hybrid with many UVs and possible asynapsis. FIG. 11. Multivalent association in the pachytene stage of meiosis in the hybrids, MV = multivalent.

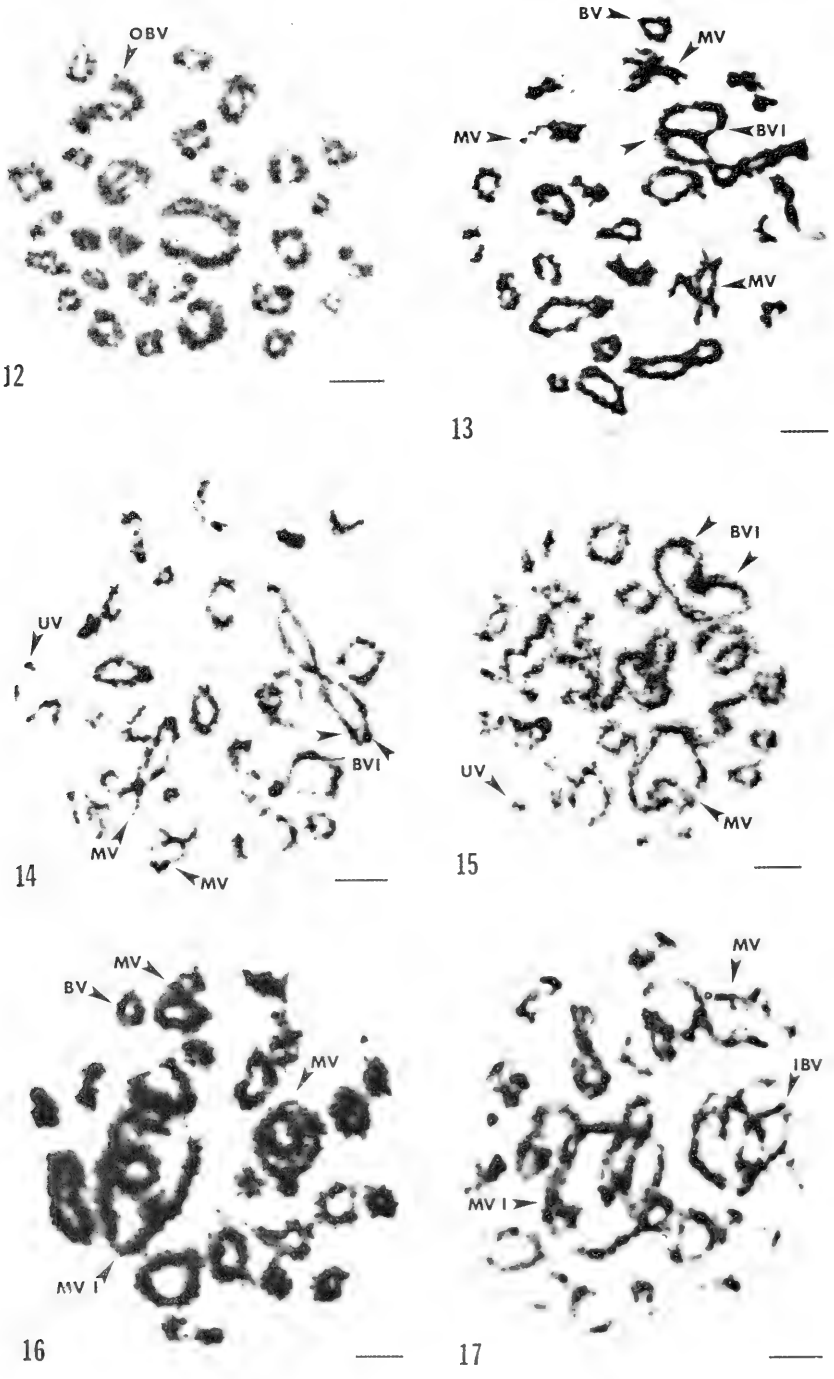

FIG. 12. Early diakinesis in the hybrid, two-by-two pairing is evident, OBV = odd bivalent. FIG. 13. Late diplotene in the hybrid, arrows point to the two ends of chromosomes not synapsing with each other; BVI = bivalent formed by the largest pair of chromosomes. FIG. 14. Late diplotene stage in the hybrid, arrows on BVI point to its heteromorphic nature. FIG. 15. Late diplotene stage of the hybrid; arrows on BVI point to the folded ends of the two chromosomes. FIG. 16. Diakinesis stage of the hybrid; MVI = multivalent complex associated with BVI. FIG. 17. Late diplotene stage of the hybrid with MVI; IBV = interlocking bivalent.

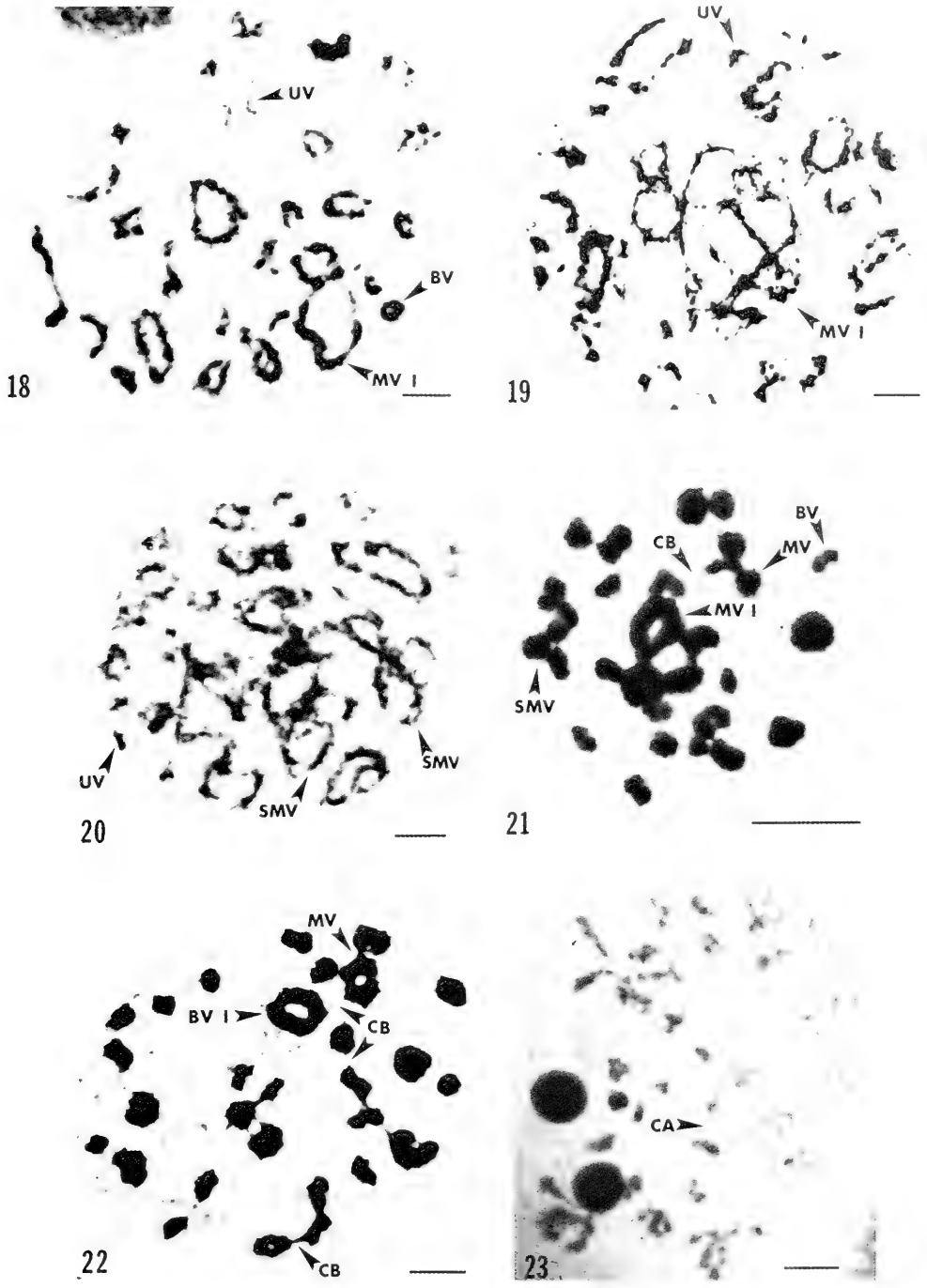

FIG. 18. Early diakinesis in the hybrid with MVI. FIG. 19. Diplotene stage in the hybrid; here MVI has at least 12 chromosomes. FIG. 20. Diplotene stage in the hybrid, with apparently sticky multivalents (SMV). FIG. 21. Metaphase I in the hybrid; CB = chromosomal bridge. FIG. 22. Metaphase I in the hybrid, with CBs, free BVI and MV. FIG. 23. Metaphase II chromosomes in the hybrid; CA = chromosomal aberration (fragment).

some interesting observations. For example, this chromosome pair may synapse in a "normal" manner resulting in a "normal" bivalent (Figs. 13–15), or may form complex multivalents (Figs. 16–22). The multivalent complexes of 6–14 or more chromosomes encountered in the hybrids indicate that a number of reciprocal chromosomal exchanges have occurred between the largest chromosome (no. 1) and others during the course of evolution of each parental species. Thus, the two largest chromosomes behave as "homologues" in the meiosis of each parent (Figs. 7, 8), due to their obvious homozygosity. This would also explain the occurrence of multivalent complexes in the meiosis of the hybrids. The number of chromosomes in a multivalent association can be analogized to a chain with many links. If one link is missing, then two shorter chains would be formed instead of a long one. The recognition of all the translocated segments in the hybrid meiosis did not occur all the time (Table 1 and Figs. 12-15).

The non-homologous nature of parts of the bivalent no. 1 is evident during a close examination due to (i) failure to synapse completely resulting in an interesting bivalent whose two ends are separate (Fig. 13), (ii) different morphology of each chromosome in the bivalent with lack of chiasmata in the non-homologous regions (Fig. 14), or (iii) thickening of such regions of chromosomes when a single bivalent was encountered (Fig. 15). It is therefore concluded that the morphology of the bivalent formed by the largest chromosome pair (no. 1) is suggestive of its partially non-homologous nature, and the multivalents associated with it are not artifacts. Such multivalent associations were

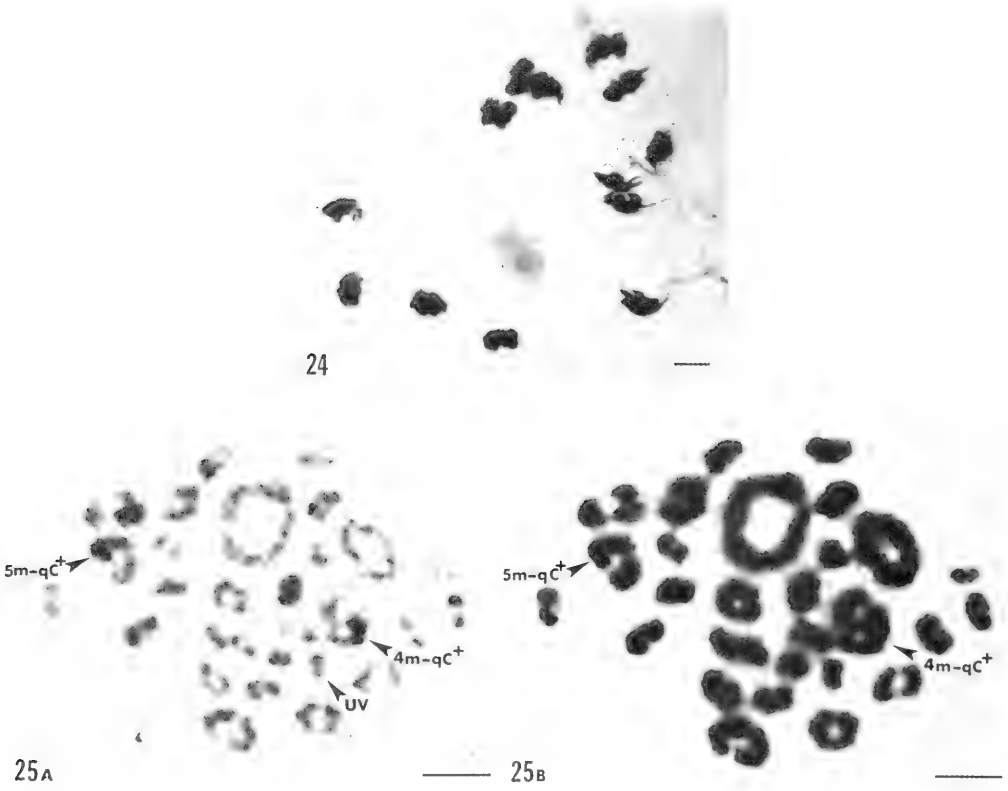

FIG. 24. Anaphase II cells in the hybrid, becoming sticky and abortive. FIG. 25A. The same chromosome spread after C-banding treatment. Here 4 *m*–q C + and 5 *m*–q C + of *A. p. albicauda* are seen synapsing with 4 *m* and 5 *m* of *A. lenticula*; the lack of chiasmata between the C + regions of *A. p. albicauda* marker chromosomes with the euchromatic ones of *A. lenticula* is evident. FIG. 25B. Late diakinesis chromosome spread of the hybrid stained with Giemsa.

frequently encountered in other chromosomes, too (Figs. 13–20). Interlocking and sticky bivalents could also be inferred in some meiotic preparations of the hybrids (Figs. 17, 20–22).

Chromosomal aberrations (e.g. sticky chromosomes, fragments, etc.) in metaphase II of meiosis were also discovered (Fig. 23), indicating that they were a result of an abnormal prophase I and metaphase I of meiosis being passed on to the secondary spermatocytes. Some sperm was also found in the squash preparations of the hybrid ovotestes. However, it is very unlikely that a significant number of such sperm were without chromosomal aberrations, i.e. genetic duplication or deficiencies. The presence of chromosomal fragments in metaphase II (Fig. 23) would support such a view. Some of the telophase II nuclei that would normally undergo spermiogenesis, were observed to become sticky and lysed (Fig. 24).

In order to demonstrate that the haploid chromosome sets of *A. p. albicauda* and *A. lenticula* are indeed involved in the meiotic process of the hybrids, we applied the C-banding technique to them. One hundred meiotic chromosome spreads of the hybrids were stained with Giemsa and recorded on photographic film. They were then destained and subjected to the C-banding technique. The rate of success was about 5%. Fig. 25B is an early metaphase I of meiosis in the hybrids. The same chromosome spread (Fig. 25A) after induction of C-bands demonstrates the *A. p. albicauda* 4 *m*–q C+ and 5 *m*–q C+ chromosomes synapse with 4 *m* and 5 *m* of *A. lenticula*. Furthermore, the morphology of the chromosomes is also clearer than before (Fig. 25B).

Conserved chromosome numbers have been reported in many groups of land snails (Patterson, 1969; Patterson & Burch, 1978; Burch, 1965; Babrakzai *et al.,* 1974). For example, the genus *Ashmunella* has a haploid chromosome number of 29 in all species studied so far (Reeder & Miller, 1974; Reeder, 1975; Reeder *et al.,* 1975; Babrakzai, in press). The standard karyotype here does add useful information to the taxonomic relationships of a species. However, such karyotypes are only starting points at best. We have encountered "similar"-looking chromosome pairs when comparing karyotypes of species of *Ashmunella* using orcein or Giemsa staining alone, which, in many instances, turned out to be quite different in their constitutive heterochromatin distribution. Therefore, even though the standard karyotype is a very useful tool in elucidating taxonomic and evolutionary relationships, it should be supplemented by the inherent morphology of the chromosomes as revealed by the C or G bands, etc. (Babrakzai & Miller, 1975; Babrakzai *et al.,* 1974, 1975a, 1976a, 1980; and Babrakzai, 1982).

The study of meiosis in the interspecies hybrids of *Ashmunella* revealed that small chromosomal exchanges that have occurred during the evolution of closely related species can be easily demonstrated since spermatogonial meiosis can be analogized with construction of precise karyotypes by the diploid gonadal cells *before* the formation of gametes. The study was complicated, however, by the fact that two-by-two bivalents were indeed observed in the hybrids, resulting in "normal" meiosis (Fig. 12 and Table 1). Thus, superficially, the explanations described above for multivalent formation lose their strength leading one to a paradoxical situation. We hypothesize that this may not be the case here. For example, genes causing isolation barriers, e.g. hybrid breakdown, etc., have been reported and postulated to be located on rearranged chromosome arms (Sanyo & Kita, 1978). These authors have proposed that "the fixation of such genes is promoted when they are linked with the breakage points of rearranged arms." Factors located on sex chromosomes have been proposed to regulate the viability of hybrids between *Drosophila hydei* and *D. neohydei* (Schaefer, 1979), and the hybrid male sterility in a cross of two species of bulls, i.e. *Bos javanicus* and *B. taurus typicus* (Steklenev & Nechiporenko, 1979). Jouve and co-workers have reported that the desynaptic effect in *Triticale* × *Secale cereale* hybrids is due to genetic differences in pairing regulators or "pairing genes" (Jouve *et al.,* 1980). Such pairing regulators may be involved in the meiosis of interspecies hybrids of leopard frogs, where pairing almost always fails and there is a high incidence of univalents (Babrakzai *et al.,* 1978). In the case of interspecies hybrids of *Ashmunella,* the possibility of such genes cannot be ruled out. The occurrence of normal meiosis could be the result of either (i) favorable expression of pairing genes in the hybrids suppressing recognition of chromosomal exchanges, or (ii) lack of chiasma formation by chance in the exchanged region of chromosomes, or both.

The incidence of chromosomal aberrations in pulmonate land snails from the arid regions of the American Southwest is not an uncommon phenomenon. Such aberrations are known from populations of *Ashmunella chiricahuana* (Polygyridae), *Radiocentrum clappi* (Oreohelicidae), and *Sonorella virilis* (Helminthoglyptidae), all from the Chiricahua Mountains of Arizona (Babrakzai *et al.,* 1975b, 1976b). Therefore, we hypothesize that at some time in the past, the pro-*proximalenticula* species population of *Ashmunella* was distributed in the Horseshoe Canyon and Jhus Canyon of the Chiricahua Mountains of Arizona. Later on, the snails became restricted to each canyon without much chance of migration back and forth due to xeric conditions outside these canyons resulting in two isolated populations. During extremely dry conditions, the species populations were reduced to very few individuals and chromosomal aberrations occurred as a result of "environmental stresses," (i.e. prolonged periods of estivation and accumulation of metabolic wastes). The deleterious chromosomal aberrations were lost by selection. On the other hand, if a new translocation or an inversion had a selective advantage, it became homozygous, or chiasma formation was suppressed due to some possible heterochromatinization in such regions of chromosomes. Another possibility is that translocation and inversion homozygosities could have been achieved by chance alone. We are inclined to think that both selection and chance probably had their roles in the evolution of karyotypes of these species. The *Ashmunella proxima* species group from the Chiricahua Mountains of Arizona has a distribution of heterochromatin in their karyotypes with interesting evolutionary implications, which will be communicated in a future article.

The reflection of all the cytological and genetic differences between *A. p. albicauda* and *A. lenticula* is also simplified in their phenotypes (Figs. 1–3). Thus, the intermediate nature of the hybrid snail shells is due to the expression of one-half of the genes from each parent. This observation also emphasizes the importance of such seemingly small, but consistent, characteristics in the shell morphology in the taxonomy of *Ashmunella*. We, therefore, suggest caution in synonymizing species of this genus.

From the foregoing, it is apparent that although *A. p. albicauda* and *A. lenticula* can be made to hybridize under laboratory conditions, the hybrids fail to undergo successful gametogenesis. This is a clear indication of reproductive isolation. W. O. Gregg's intuitive determination that *A. lenticula* was a distinct species has been supported by the results of our investigation. While this does not preclude that *A. lenticula* may not be reproductively isolated from *A. angulata,* as presumed by Bequaert & Miller (1973), we are now of the opinion that minor morphological differences in shell characters are but "the tip of the iceberg." The chromosomal differences in the two species investigated are obviously considerable in order to cause the extensive meiotic abnomalities that we observed. It can now be suspected that other species of *Ashmunella* that appear to differ only in small morphological characters have also developed sizeable chromosomal rearrangements and/or mutations sufficient to effect reproductive isolation. Accordingly, we believe that *A. lenticula* is indeed a good species and that Bequaert & Miller (1973) erred in "intuitively" synonymizing it with *A. angulata.* Our unpublished work on the interspecies hybrids of *A. proxima* and *A. angulata* indicates that these two are good species as proposed by Pilsbry (1940).

CONCLUSIONS

1. *Ashmunella proxima* and *A. lenticula* are two closely related but cytologically and genetically different species.
2. The karyotypes of the two species have evolved in different directions.
3. The observed cytological differences in karyotypes of the two species may represent a gross oversimplification of their genetic differences.
4. The incidence of observed normal synapsis in the hybrids may be due to a favorable expression of *pairing genes* of the two genomes suppressing multivalent and univalent formation in meiosis or lack of chiasmata due to chance, or both.
5. Conservatism in synonymizing land snails species of the arid Southwest is strongly recommended.

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REFERENCES CITED

BABRAKZAI, N., in press, Karyotype analysis of *Ashmunella rhyssa* complex (Gastropoda: Pulmonata: Polygyridae). *Transactions of the Missouri Academy of Science.*

BABRAKZAI, N., FROST, J. S., BAGNARA, J. T. & WARD, O. G., 1978, Meiosis in interspecies hybrids of leopard frogs. *Mammalian Chromosome News Letter,* 19: 11.

BABRAKZAI, N. & MILLER, W. B., 1974, A colchicine hypotonic squash technique for the chromosome spreads of pulmonate land snails. *Malacological Review,* 7: 37–38.

BABRAKZAI, N. & MILLER, W. B., 1975, Karyotypic comparisons between Helminthoglyptidae and Bradybaenidae (Gastropoda: Pulmonata). *Bulletin of the American Malacological Union for 1975,* p. 67.

BABRAKZAI, N., MILLER, W. B. & WARD, O. G., 1974, Cytotaxonomy of some Arizona Oreohelicidae (Gastropoda: Pulmonata). *Bulletin of the American Malacological Union for 1974,* p. 4–11.

BABRAKZAI, N., REEDER, R. L. & MILLER, W. B., 1975a, Chromosomal aberrations in Southwestern Pulmonate gastropods. *Journal of the Arizona Academy of Science, Supplement,* p. 30–31.

BABRAKZAI, N., SAMSAM, S. & MILLER, W. B., 1976a, Methods and procedures in molluscan cytology and cytogenetics. *In:* Symposium: Current trends in Malacology. *Bulletin of the American Malacological Union for 1976,* p. 57–62.

BABRAKZAI, N., SAMSAM, S. & MILLER, W. B., 1976b, Chromosomal aberrations in *Sonorella virilis* (Helminthoglyptidae: Pulmonata). *Annual Report of the Western Society of Malacologists for 1976,* p. 45.

BABRAKZAI, N., SAMSAM, S. & MILLER, W. B., 1980, Cytological relationships of Helminthoglyptidae and Bradybaenidae (Mollusca: Pulmonata). *Journal of the Arizona-Nevada Academy of Science, Supplement,* 21: 5.

BABRAKZAI, N., WARD, O. G. & MILLER, W. B., 1975b, The introduction of Giemsa and Centromeric banding techniques of chromosomes to molluscan cytotaxonomy. *Bulletin of the American Malacological Union for 1975,* p. 67.

BEQUAERT, J. C. & MILLER, W. B., 1973, *The mollusks of the arid southwest.* University of Arizona, Tucson, Arizona: i–xvi + 271 p.

BURCH, J. B., 1965, Chromosome numbers and systematics in Euthyneuran snails. *Proceedings of the First European Malacological Congress:* 215–241.

CHRISTENSEN, K. & SEEDEGARD, K., 1979, Chromosome markers in domestic pigs. *Hereditas,* 90: 303–304.

CRIBIU, E. P. & DE GIOVANNI, A. M., 1978, The C-banding pattern of the domestic horse (*Equus caballus*), the donkey (*Equus asinus*), and the mule. *Annales de Génétique et de Selection Animale,* 10: 161–170.

JOUVE, N., DIEZ, N. & RODRIGUEZ, M., 1980, C-banding in *6X-Triticale* × *Secale cereale* hybrid cytogenetics. *Theoretical and Applied Genetics,* 57: 75–79.

LIMING, S., YINGYING, Y. & XINGSHENG, D., 1980, Comparative cytogenetic studies on the red muntjack (*Montiacus muntjack vaginalis*), Chinese muntjack (*Montiacus reevesi*), and their F_1 hybrids. *Cytogenetics and Cell Genetics,* 26: 22–27.

MANCINO, G. M., RAGGIANTI, M. & BUCCI-INNOCENTI, S., 1979, Experimental hybridization within the *Triturus* (Urodela: Salamandridae), 3; Evidence for crossingover, true chiasmata and chromosomal homologies in spermatogenesis of F_1 species hybrids *Triturus cristatus carnifex* female × *T. marmoratus. Chromosoma,* 73: 207–226.

McKRACKEN, G. F. & BRUSSARD, P. F., 1980, The population biology of the white lipped land snail, *Triodopsis albolabris* and its genetic variability. *Evolution,* 34: 92–104.

PATTERSON, C. M., 1969, Chromosomes of Molluscs. *Proceedings of the Symposium on Mollusca Part II, Marine Biological Association,* India, p. 636–686.

PATTERSON, C. M. & BURCH, J. B., 1978, Chromosomes of pulmonate molluscs. *In: Pulmonates,* FRETTER, V. & PEAKE, J., eds., Academic Press, p. 171–217.

PILSBRY, H. A., 1940, Land Mollusca of North America (north of Mexico). *Academy of Natural Sciences of Philadelphia, Monograph* 3 (Vol. I, part 2); i + viii + 575–994 + i–ix p.

REEDER, R. L., 1975, *Comparative karyotype analyses of selected members of the genus* Ashmunella *(Mollusca: Pulmonata: Polygyridae).* Ph.D. Thesis, University of Arizona, 75 p.

REEDER, R. L. & MILLER, W. B., 1974, Cytotaxonomy of some Arizona *Ashmunella* (Gastropoda: Pulmonata). *American Zoologist,* 14: 1264.

REEDER, R. L., BABRAKZAI, N. & MILLER, W. B., 1975, Cytotaxonomic study in Polygyridae (Gastropoda: Pulmonata). *Journal of the Arizona Academy of Science, Supplement,* p. 15.

SANYO, Y. & KITA, F., 1978, Genes for reproductive isolation located on rearranged chromosomes. *Heredity,* 41: 377–389.

SCHAEFER, U., 1979, Viability in *Drosophila hydei* × *D. neohydei* and its regulation by genes located in the sex heterochromatin. *Biologisches Zentralblatt.* 98: 153–161.

SHCHAPOVA, A. I. & ZARIPOVA, Z., 1979, Identification of rye chromosomes in the karyotypes of wheat-rye hybrids of Giemsa C-banding Techniques. *Izvestiya Sibirskogo Otdeleniya Akademii Nauk SSSR Seriya Biologicheskikh Nauk,* 0(2): 85–90.

STEKLENEV, E. P. & NECHIPORENKO, V. K., 1979, Chromosomal complexes of hybrids between *Bos (Bibos) javanicus* and *Bos (Bibos) taurus typicus. Tsitologiya Genetika,* 13: 31–33.

SUMNER, A. T., 1972, A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research,* 57: 304–306.

TRIFAN, D. M., 1976, *A comparative electrophoretic study of several* Ashmunella *populations from the Chiricahua Mountains of Arizona.* Master's Thesis, University of Arizona, 27 p.

YOSIDA, T., 1979, Genetic aspects of polymorphic C-bands in the chromosomes of the black rat *(Rattus rattus tanezumi). Cytologia (Tokyo).* 44: 265–274.

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CHROMOSOMAL EVOLUTION IN PLANORBID SNAILS OF THE GENERA *BULINUS* AND *BIOMPHALARIA*

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ABSTRACT

The planorbid snail genera *Bulinus* and *Biomphalaria* have presented the systematist with problems of critical interest. The basic chromosome number for the family Planorbidae is $2n = 2x = 36$, but species of *Bulinus* have been shown to exhibit four levels of ploidy—diploid, tetraploid, hexaploid and octoploid. In contrast, none of the many populations of *Biomphalaria* that have been studied cytologically in this and in previous reports contain other than the basic diploid chromosome number.

In the present paper, we review the data on karyotypic evolution in *Bulinus* and *Biomphalaria*, and data relevant to a description of the breeding biology of these two groups. We then discuss the possibility of a connection between breeding biology and chromosomal evolution in *Bulinus* and *Biomphalaria*, in order to assess the validity and applicability of models proposing a connection between inbreeding or low effective population size and rapid rates of chromosomal evolution.

Our data revealed that *Biomphalaria* is karyotypically more conservative than *Bulinus* at the diploid level. While many species of *Bulinus* differ in karyotype, *Biomphalaria* species are not distinguishable by karyotype analysis. We studied G-banded karyotypes of four populations of *Biomphalaria glabrata* and one of *B. straminea, Bulinus tropicus, B. natalensis, B. truncatus* and *B.* sp. ($2n = 36$) from Mazoe Dam, Zimbabwe. In considering these data, and data on nine species published by previous workers, we observed from 14 to 16 metacentric pairs in all *Biomphalaria* species studied, while the corresponding range for *Bulinus* was from 8 to 17.

We hypothesize that the contrasting rates of chromosome evolution between *Bulinus* and *Biomphalaria* is a result of *Bulinus* having small effective population size as compared with *Biomphalaria*.

Key words: *Bulinus; Biomphalaria;* polyploidy; cytogenetics; karyotypes; evolution; breeding system.

INTRODUCTION

It is widely held that most species differ from one another in some detectable aspect of karyotype, although exceptions are certainly known (White, 1978). The extent and quality of divergence at the chromosome level varies and depends, in part, upon the breeding biology of the organisms concerned.

We examine the extent of karyotypic divergence within and among species of the planorbid snail genera *Bulinus* and *Biomphalaria*, in order to determine how useful cytogenetics may be as a tool to study taxonomic problems and evolutionary processes. We then review briefly the extent of karyotypic divergence found in various animal and plant species which differ in breeding biology. We subsequently discuss the possibility of a connection between breeding biology and chromosomal evolution in *Bulinus* and *Biomphalaria*, in order to assess the validity and applicability of models proposing a connection between inbreeding or low effective population size and rapid rates of chromosomal evolution.

The planorbid snail genera *Bulinus* and *Biomphalaria* have presented the systematist with problems of critical interest. These genera include the freshwater snails which serve as the obligate intermediate host for three species of *Schistosoma,* the etiologic

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agent for human schistosomiasis, a disease of major world health importance (Ansari, 1973). In many cases, those species which do transmit schistosomiasis are no different morphologically from those which do not, while they may be distinguishable on the basis of biochemical or cytological characters (Brown, 1980). The basic chromosome number for the family Planorbidae is $2n = 2x = 36$, but species of *Bulinus* have been shown to exhibit four levels of ploidy—diploid, tetraploid, hexaploid, and octoploid (Patterson & Burch, 1978). In contrast, all of the populations of *Biomphalaria* which have been studied cytologically have the basic diploid chromosome number.

The state of the art in pulmonate snail cytogenetics was recently reviewed in detail by Patterson & Burch (1978). It is not meaningful, at this point, to discuss the rate of karyotypic evolution in the pulmonates as a group, because few species have actually been karyotyped (Patterson & Burch list 26 species). Extensive work is, however, underway with land snails of the genera *Ashmunella* (Babrakzai *et al.*, 1974, and personal communication) and *Cepaea* (Page, 1978; Gill & Cain, 1980).

Reliable chromosome counts, however, are available on many pulmonate groups (Patterson & Burch, 1978). The observed range is from $2n = 10$ to $2n = 144$; omitting the known or suspected cases of polyploidy reduces the upper limit to $2n = 88$. Within lower taxonomic categories, such as the family Planorbidae, chromosome numbers are quite conservative, $2n = 36$ throughout this family, with the exception of the polyploid *Bulinus* species.

MATERIALS AND METHODS

Snail Stocks

Table 1 summarizes all snail stocks used or referenced in this paper. The table refers the reader to previous publications for further de-

TABLE 1. *Bulinus* or *Biomphalaria* populations referenced or studied.

Species	Locality or strain	Reference for collection data
Biomphalaria glabrata (Say)	NIH 6-4-1	this report
	NIH 10R2	this report
	Dominican Republic	this report
	Brazil	this report
	Brazil	Narang (1974)
	St. Lucia	this report
	Puerto Rico	Raghunathan (1976)
Biomphalaria straminea (Dunker)	Brazil	this report
Biomphalaria tenagophila (Orbigny)	Argentina	Giacomozzi *et al.* (1979)
Bulinus tropicus (Krauss)	Onderstepoort, Transvaal, South Africa	Wu (1972) and this report
	Mazoe Dam, Salisbury, Zimbabwe	this report
Bulinus natalensis (Kuster)	Nelspruits, Transvaal, South Africa	Schutte (1966) and this report
	Durban, South Africa	Claugher (1971)
Bulinus truncatus (Audouin)	Luxor, Egypt	this report
	Ferras Canal, Iraq	Claugher (1971)
Bulinus obtusispira (Smith)	Madagascar	Claugher (1971)
Bulinus liratus (Tristram)	Basibasy, Madagascar	Claugher (1971)
Bulinus globosus (Morelet)	Ombeyi Swamp, Kenya	Claugher (1971)
Bulinus forskalii (Ehrenberg)	Awash Valley, Ethiopia	Claugher (1971)

scription. Where no other publication has been referenced, the stocks are described in additional detail below.

Biomphalaria glabrata/6-4-1 is a National Institutes of Health (NIH) stock susceptible to an NIH Puerto Rican strain of *Schistosoma mansoni* (Trematoda: Digenea). The stock is marked with the recessive "albino" mutation, and originated at least ten years ago from a Puerto Rican sample. It was received at Purdue in 1976 from Dr. C. S. Richards, NIH, Bethesda, Maryland.

Biomphalaria glabrata/10R2 is a similar stock but is not susceptible to the Puerto Rican strain of *S. mansoni* mentioned above. It, too, was received from Dr. Richards.

Biomphalaria glabrata/Dominican Republic was originally collected by Dr. J. F. Maldonado in 1974 at the Cuaron River and Swamp, Los Cedros, Dominican Republic. It was sent to Purdue in 1981 by Dr. Richards.

Biomphalaria glabrata/Brazil was collected in 1974 by Dr. M. Radke, U.S. Army Medical Corps, from Parana, Curitiba, Brazil, and was supplied by Dr. Richards in 1981. The founding population at NIH consisted of only five snails.

Biomphalaria glabrata/St. Lucia was also supplied by Dr. Richards. Its exact origin is unknown, but it was collected in St. Lucia at least ten years ago.

Biomphalaria straminea was collected by Dr. W. L. Paraense in 1971, at Sete Lagoas, Minas Gerais, Brazil. It was supplied to us by Dr. Richards in 1981.

Bulinus sp. ($2n = 36$)/Mazoe was collected in 1981 by Mrs. F. Matthews, Blair Research Institute, Salisbury, Zimbabwe. The snails were collected at Mazoe Dam, 40 km from Salisbury. The snails have only provisionally been identified as *B. tropicus*.

Snail Rearing

Snails used in this study were maintained according to the methods outlined in Goldman *et al.* (1980).

Slide Preparation and Banding

Air-dried preparations were made and G-banded (ASG technique) as outlined in Goldman *et al.* (1983a). Ag-NOR banding (Howell & Black, 1980) to reveal nucleolar organizer regions (NOR's) was done on unstained slides which had been incubated in a dry oven at 40°C overnight, then counterstained for 14 seconds with 1:50 Gurr's improved R66 Giemsa in Gurr's buffer, pH 6.8. NOR-banded slides for analysis were photographed as outlined in Goldman *et al.* (1983b), but the most satisfactory reproduction of these slides was obtained by photography on Kodak tungsten Ektachrome ASA 160 color slide film without a filter.

Karyotyping and Data Analysis

The procedures for arranging karyotypes and analyzing data were described fully in Goldman *et al.* (1983a).

Criteria for grouping *Bulinus* chromosomes were as detailed in Goldman *et al.* (1983a). *Biomphalaria* chromosomes were grouped as follows. Group I contained the largest pair, usually metacentric. The next two chromosomes, in decreasing order by size, were classified in Group II and were generally metacentric. Group III was composed of the next three chromosomes, which were noticeably less metacentric than Group II chromosomes, but similar in size. Group IV was consistently the single, large, subtelocentric pair. Group V generally contained three metacentric chromosomes, smaller than those found in Groups II and III. Group VI consistently contained the chromosome pair with the lightly-staining arm. Group VII was similar in size to Group V, but the chromosomes generally had a more terminal centromere. Frequently, however, it was difficult to distinguish Groups V and VII. Group VIII generally contained one pair with the centromere nearly terminal in position, and was always classified as a submetacentric pair. Group IX was usually composed of the two smallest pairs in the karyotype; these were metacentric.

Mean relative chromosome length and arm ratio, an index of centromere position, were calculated for two to twelve cells of each population and the data with standard errors are reported in Appendices 1–6. It should be noted that some of the variability apparent in the measurements is due to the observer's failure to recognize, for instance, chromosome 4 as distinct from chromosome 5 in every *Bulinus tropicus* cell. Only distinctive chromosomes, such as the LSA pair and the subtelocentric pair, can be reliably selected for measurement every time. It should further be noted that interpopulation comparisons of these data are not strictly valid, because a change in the size of one chromosome, as by loss or duplication of heterochromatin, would result in a change in the relative chromosome

length of all chromosomes in the karyotype. Comparing similarly numbered groups of different species, then, does not necessarily mean comparing chromosomes of common descent. This problem can only be overcome with refined banding procedures, which should allow the identification of individual, homologous chromosomes or chromosome segments in every species.

Chromosomes from published karyotypes were grouped according to the same criteria used for grouping chromosomes for karyotypes prepared in this research. The numbers of pairs falling into various groups were then compared with the summary data in Table 2 in order to assess similarities and differences between the published karyotypes and those reported here for the first time.

Measurements were obtained for the idiograms of Claugher (1971). Chromosomes were measured, then grouped in order to see if the groupings corresponded with the chromosome groups obtained for similar material prepared in this laboratory. Group mean measurements were calculated (see Goldman *et al.,* 1983a) for documentation of the results.

RESULTS

Figs. 1–6 show representative karyotypes of the six populations of *Biomphalaria* studied, and of *Bulinus* sp. ($2n = 36$) from Mazoe Dam, which is provisionally identified as *Bulinus tropicus.* Relative chromosome measurements and arm ratios are given in Appendices 1-6. Comparable data for the *Bulinus* population reported previously are found in Goldman *et al.* (1980) for *B. tropicus* and *B. natalensis* and Goldman *et al.* (1983a) for *B. truncatus.*

The most conspicuous features of the karyotypes were the subtelocentric pair (Group IV) present in all populations of *Biomphalaria* (Figs. 1–5) and absent from the Mazoe Dam population (Fig. 6) and other populations of *Bulinus* which have been studied (Goldman *et al.,* 1980, 1983a). A lightly-staining arm (LSA) pair, reported previously (Goldman *et al.,* 1980, 1983a), was evident in all populations studied here, and has been placed in Group VI.

We reported (Goldman *et al.,* 1983a) for *Bulinus tropicus* and *Bulinus truncatus* that the entire lightly-staining arm of the LSA pair in Group VI chromosomes stains positively as

FIG. 1. Karyotype for *Biomphalaria glabrata,* NIH strain 6-4-1. Bar is 5 μm.

an Ag-NOR (nucleolar organizer region) when slides were treated according to the procedure of Howell & Black (1980). We have repeated the procedure on four stocks of *Biomphalaria glabrata*. In every case, it was clear that the short arm of the LSA pair stained positively. There was some evidence for other pairs of chromosomes staining positively in a well-defined, terminal dot in some but not in all preparations. For the present, we consider only the LSA short-arm regions to be positively-staining NOR's. These results are discussed in detail in Goldman *et al.* (1983b).

Comparison of the karyotypes is facilitated by the summary table (Table 2) in which are listed the number of chromosomes in each

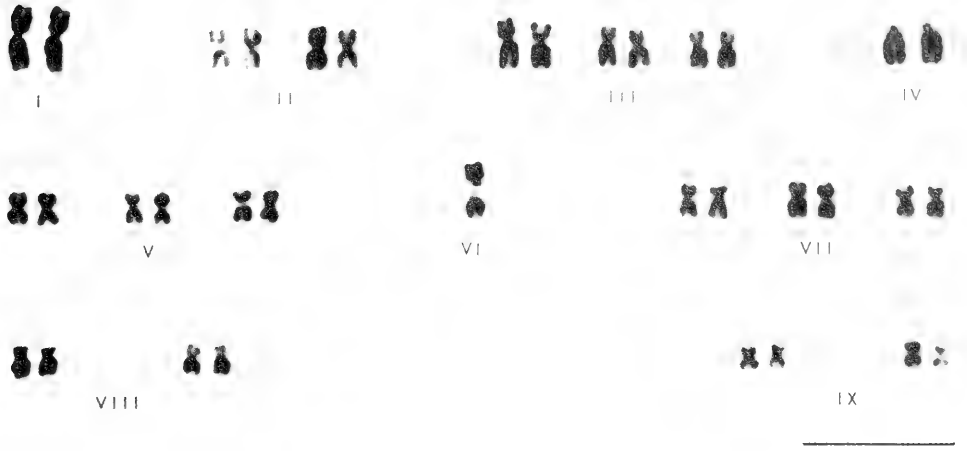

FIG. 2. Karyotype of *Biomphalaria glabrata*, NIH strain 10R2. Bar is 5 μm.

FIG. 3. Karyotype of *Biomphalaria glabrata* from the Dominican Republic. Bar is 5 μm.

group, the shape (centromere position) classification of that group according to the criteria of Levan *et al.* (1964), and the number of pairs found in that group for all populations reported here. Populations previously reported by this laboratory are also listed, including *Bulinus natalensis* and *Bulinus tropicus* (Goldman *et al.,* 1980) and *Bulinus truncatus* (Goldman *et al.,* 1983a). As *Bulinus truncatus* is a tetraploid, the number of pairs in each group is expected to be twice that in corresponding groups of diploid *Bulinus.*

It is apparent from Table 2 that there were no karyotypic differences among the 6-4-1, 10R2, and Brazil stocks of *Biomphalaria glabrata,* each of which had 15 metacentric, 2 submetacentric, and 1 subtelocentric pair. The Dominican Republic material differs in

FIG. 4. Karyotype of *Biomphalaria glabrata* from Brazil. Bar is 5 μm.

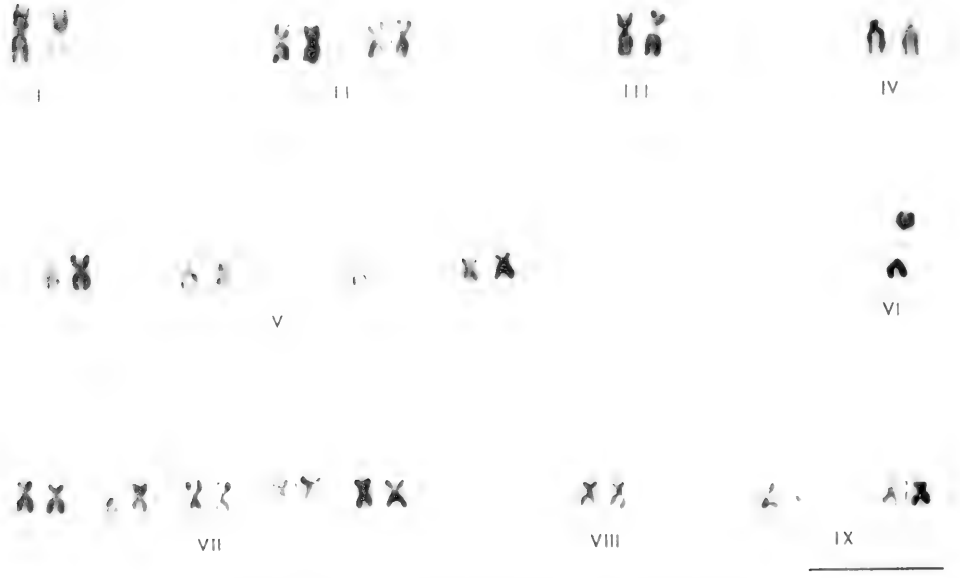

FIG. 5. Karyotype of *Biomphalaria straminea.* Bar is 5 μm.

FIG. 6. Karyotype of *Bulinus* sp. ($2n$ = 36) from Mazoe Dam, Salisbury, Zimbabwe. Bar is 5 μm.

TABLE 2. Chromosome morphology classification[1] and groupings for all *Bulinus* and *Biomphalaria* taxa for which original data are presented here.

Group no.	Population designation								
	Biomphalaria glabrata				*Biomph. straminea*	*Bulinus* Mazoe	*Bulinus tropicus*	*Bulinus natalensis*	*Bulinus truncatus*
	6-4-1	10R2	D.R.	Brazil					
I	1 m	1 m	1 m	1 m	1 m	1 m	1 m	1 m	2 m
II	2 m	2 m	2 m	2 m	2 m	1 m	1 m	1 m	2 m
III	3 m	3 m	1 m	3 m	1 m	1 m	1 m	1 m	2 m
IV	1 st	1 st	1 st	1 st	1 st	2 m	2 m	2 m	5 m
V	3 m	3 m	4 m	3 m	4 m	2 sm	2 sm	2–3 m	4 sm
VI	1 sm	1 sm	1 sm	1 st	1 sm	1 sm	1 sm	1 sm	1 m
VII	4 m	3–4 m	3 m	4 m	5 m	4 m	4 m	2 m	7 m
VIII	1 sm	1–2 sm	2 sm	1 sm	1 m	2 m	4 m	2–3 m	8 m
IX	2 m	2 m	3 m	2 m	2 m	3 sm	1 sm	3 m	2 sm
X	—	—	—	—	—	1 m	1 m	2 m	1 st
XI	—	—	—	—	—	—	—	—	2 m
Totals									
m	15	14–15	14	15	16	12	14	17	29
sm	2	2–3	3	2	1	6	4	1	6
st	1	1	1	1	1				1
No. of cells scored	8	10	9	8	2	7	12	9	9

[1]m = metacentric, sm = submetacentric, st = subtelocentric.

having 14 metacentric, 3 submetacentric, and 1 subtelocentric pair. These differences arise in Group II, which had two fewer metacentric pairs than did the modal karyotype, in Group VIII, which had one more submetacentric pair, and in Group IX, which had one more metacentric pair than usual. Groups V and VII have also exchanged one pair, either group being classified as metacentric; the importance of this is questionable because in many preparations it is not possible to unequivocally distinguish groups V and VII.

In the Brazil population of *Biomphalaria glabrata* (Fig. 4), Group VI was scored as subtelocentric instead of metacentric. This group comprises the LSA pair; the short arm was variable in length. The observed shape difference is, therefore, unimportant.

We were able to prepare only one karyotype of *Biomphalaria glabrata* from St. Lucia, and that material was of limited quality. The large subtelocentric pair seems to be present. The LSA pair was not readily recognized, but its presence could not be excluded because of the state of condensation of the chromosomes.

Unfortunately, we were unable to examine more than two karyotypes of *Biomphalaria straminea* (Fig. 5). They differed from the usual *Biomphalaria glabrata* karyotype in lacking two metacentrics in Group III, which appear instead in Groups V or VII. Groups V and VII could not be distinguished on the basis of the small amount of material available for *Biomphalaria straminea.* Group VIII was scored as metacentric instead of submetacentric. This difference is unlikely to be important, since Group VIII was near the class limits for "metacentric" in *Biomphalaria straminea.* The exact value reported, in view of the small sample size, may be imprecise.

Karyotypic differences were apparent among the populations of *Bulinus* studied. We have presented evidence (Goldman *et al.,* 1980) for karyotypic differentiation between *Bulinus tropicus* and *Bulinus natalensis,* two diploid species which differ in susceptibility to parasite infection, in some aspects of biochemistry and immunology, and in the frequencies of some morphological characters (for details and references, see Goldman *et al.,* 1980). The summary table (Table 4) shows that *Bulinus tropicus* had 14 metacentric and 4 submetacentric pairs, while *Bulinus natalensis* had 17 metacentric and 1 submetacentric pair. Differences were apparent in Groups V, VII, VIII, IX and X. The measurements for Group IX, scored as submetacentric in *Bulinus tropicus* and as metacentric in *Bulinus natalensis,* were substantially different, though the submetacentric group did lie close to the class limits for reclassification as "metacentric."

There was presumptive evidence for karyotypic differentiation between Groups VIII and IX of the two populations of *Bulinus tropicus. Bulinus tropicus* from Onderstepoort had two more Group VIII chromosomes while *Bulinus* sp. ($2n = 36$) from Mazoe Dam had two more Group IX chromosomes.

Further evidence of karyotypic change in *Bulinus* was provided by the tetraploid, *Bulinus truncatus,* which could indicate that this species arose through hybridization in which one of the parents lacked the LSA feature. Secondly, there was only one subtelocentric pair in *Bulinus truncatus* (Group X), suggesting a hybrid origin from a population of diploid *Bulinus* which did have such a feature. The subtelocentric pair is not likely to be the "unmatched" pair from Group VI, since the long arms in each case were not similar in size, unless we assume that additional rearrangements involving these pairs have occurred (Goldman, 1981). Thirdly, we have observed pairs of, as opposed to sets of four, satellited chromosomes which did not occur in any of the diploid populations studied, again suggesting a hybrid origin involving a diploid karyotypically dissimilar to *Bulinus tropicus.* The evidence concerning the hybrid origin of *Bulinus truncatus* from Egypt was detailed in Goldman *et al.* (1983a).

DISCUSSION

Chromosomal Evolution in *Bulinus* and *Biomphalaria*

The data presented suggest that *Biomphalaria* species are more karyotypically conservative than *Bulinus* species. These findings are consistent with previous reports in the literature.

Abundant information is available on chromosome numbers of *Bulinus* and *Biomphalaria* (Patterson & Burch, 1978), yet little work has been done on karyotypes. All published karyotype work on these two genera of which we are aware is referenced and briefly described in Table 3.

The only published karyotype of *Bulinus*, other than our own (Goldman *et al.,* 1980), is

TABLE 3. Available karyotypes of Planorbid snails.

Species	Remarks	Reference(s)
Biomphalaria glabrata	good material	Narang, 1974
Biomphalaria glabrata	good material	Raghunathan, 1976
Biomphalaria glabrata	good material; 5 populations	this report
Biomphalaria tenagophila	good material	Giacomozzi *et al.*, 1979
Biomphalaria straminea	limited material	this report
Bulinus tropicus	fair material	Patterson, 1971
Bulinus tropicus	good material	this report and Goldman *et al.*, 1980
Bulinus "tropicus"	idiogram only	Claugher, 1971
Bulinus "natalensis"	idiogram only	Claugher, 1971
Bulinus "natalensis"	good material	this report and Goldman *et al.*, 1980
Bulinus liratus	idiogram only	Claugher, 1971
Bulinus truncatus	idiogram only	Claugher, 1971
Bulinus truncatus	good material	Goldman *et al.*, 1983a
Bulinus globosus	idiogram only	Claugher, 1971
Bulinus obtusispira	idiogram only	Claugher, 1971
Bulinus forskalii	idiogram only	Claugher, 1971

TABLE 4. Chromosome morphology classification[1] and grouping for seven taxa of *Bulinus*, derived from the data of Claugher (1971).

Group no.	*Bulinus obtusispira*	*Bulinus truncatus*	*Bulinus "tropicus"*	*Bulinus liratus*	*Bulinus "natalensis"*	*Bulinus forskalii*	*Bulinus globosus*
I	1 m	2 m	1 m	1 m	1 m	1 m	1 m
II	1 sm	2 sm	1 m	1 m	1 m	1 sm	1 sm
III	1 m	2 m	1 m	1 m	1 sm	1 m	1 m
IV	2 m	4 m	2 m	2 m	2 m	2 m	2 m
V	2 sm	6 m	2 m	2 sm	2 sm	3 sm	2 m
VI	—	—	—	—	—	—	—
VII	4 m	8 m	5 sm	5 m	3 m	6 sm	5 sm
VIII	5 m	6 m	4 m	4 m	2 sm	2 m	4 m
IX	—	2 sm	1 m	1 m	4 m	1 m	1 m
X	2 m	—	1 m	1 m	2 m	1 m	1 m
XI	—	4 m	—	—	—	—	—
Totals							
m	15	32	13	16	13	8	12
sm	3	4	5	2	5	10	6

[1]m = metacentric, sm = submetacentric, st = subtelocentric.

that of Patterson (1971) for *Bulinus tropicus.* Her findings are compatible with our results.

Claugher (1971) studied *Bulinus* populations including three of the four species groups currently recognized by Brown (1980)—the *truncatus/tropicus* complex, including *Bulinus tropicus, Bulinus truncatus, Bulinus natalensis* and *Bulinus liratus*; the *forskalii* group, including *Bulinus forskalii,* and the *africanus* (Krauss) group, including *Bulinus globosus* and *Bulinus obtusispira.* Since his is clearly the most extensive study of *Bulinus* karyotypes, it is unfortunate that Claugher's published material consists solely of idiograms, idealized chromosomes based on measurements of several cells. The number of cells measured for each population is not indicated, nor are the average measurements and standard errors. One must interpret Claugher's results with caution, because photographs are not presented which would allow one to critically judge the quality of the work.

It would be a serious error, however, to ignore Claugher's (1971) data, since comparable data may not be available in the near

future. We have, therefore, grouped the chromosomes shown in Claugher's idiograms according to the same criteria used in analyzing our own data. Numerical data are provided in Goldman (1981), and a summary of the data appears in Table 4.

Claugher's (1971) idiograms do not indicate the presence of an LSA pair. It is likely that the chromosomes Claugher studied were too constricted to allow recognition of this pair. In fact, our earlier work (Goldman *et al.,* 1980) on *Bulinus tropicus* and *Bulinus natalensis* made only casual reference to the LSA pair, since at that time our chromosome preparations were too constricted to allow reliable observation of this.

Claugher's (1971) data on *Bulinus tropicus, Bulinus natalensis, Bulinus forskalii* and *Bulinus globosus* are remarkable in having a large number of submetacentric chromosomes. The populations of *Bulinus tropicus* and *Bulinus natalensis* that we studied did not have large numbers of submetacentric chromosomes. It is not likely that Claugher's depiction of so many submetacentric pairs is a technical artifact; the largest pair of *Bulinus globosus,* which should always have been recognized and measured reliably, is less metacentric (lower arm ratio value) than comparable chromosomes in any of our material. This may very well indicate a translocation of material from one of the smaller metacentric pairs to the largest metacentric pair, producing two submetacentrics as a result. Claugher has only provisionally identified his material as *Bulinus tropicus* and *Bulinus natalensis,* and the referral of our "*Bulinus natalensis*" population from a different locality to that taxon has been questioned [D. S. Brown, British Museum (Natural History), personal communication]. This could account, in part, for differences in our findings.

Claugher's (1971) idiogram of the tetraploid *Bulinus truncatus* does not indicate the presence of any chromosomes appearing in pairs rather than in sets of four. This is contrary to our findings (Goldman *et al.,* 1983a). Claugher reports no satellites in large or small chromosomes, nor does he report a subtelocentric pair.

Claugher's (1971) work suggests a lack of conservatism in the chromosomal evolution of *Bulinus*. This is apparent from the wide variation among species in number of metacentric and submetacentric chromosomes demonstrated in Claugher's idiograms. These differences, moreover, are as wide within species groups as they are between them. For instance, *Bulinus obtusispira* and *Bulinus liratus* have similar karyotypes, according to Claugher's data, yet fall into the *africanus* group and *truncatus/tropicus* complex, respectively. *Bulinus globosus* and *Bulinus obtusispira,* however, differ in Group VII, yet both species are in the *africanus* group.

In contrast to our findings in *Bulinus*, in *Biomphalaria* we have found little interspecific variation in karyotype. In the five populations of *Biomphalaria glabrata* we have studied there is little karyotypic variation among populations within a species. However, we only studied two *Biomphalaria* species in sufficient detail to report, and all karyotype studies to date have been on New World rather than Old World species of *Biomphalaria*.

Reports are available in the literature for populations of *Biomphalaria glabrata* from Brazil (Narang, 1974) and from Puerto Rico (Raghunathan, 1976) and for two populations of *Biomphalaria tenagophila* from Argentina (Giacomozzi *et al.,* 1979). Raghunathan's *Biomphalaria glabrata* karyotype and the *Biomphalaria tenagophila* karyotypes are quite similar to the results presented here for *Biomphalaria glabrata*, and provide no evidence for karyotypic diversity in *Biomphalaria*. Raghunathan's (1976) karyotype seems to indicate the presence of an LSA pair (No. 9) which she interprets as a satellite that is occasionally present. Narang's (1974) results are more difficult to interpret. His *Biomphalaria glabrata* material was prepared by a squash technique, and the chromosomes appear to be quite small. He indicates a small submetacentric pair, No. 16, which is probably equivalent to the Group VI LSA pair shown in this paper. Giacomozzi *et al.* (1979) consider their karyotype of *Biomphalaria tenagophila* to be equivalent to Narang's results, and suggest that there is little interspecific karyotypic differentiation in *Biomphalaria*.

The karyotype of *Biomphalaria glabrata* from the Dominican Republic differed from the remaining populations of *Biomphalaria glabrata* studied in the numbers of chromosomes assigned to various groups. This conclusion is based on a number of specimens and is unlikely to be explained simply as error of measurement. It does not, however, give adequate cause to reject the hypothesis that *Biomphalaria* is more karyotypically conservative than *Bulinus* for the following reasons: (1) The Brazil population of *Biompha-*

laria glabrata, though geographically remote from the others, had a karyotype indistinguishable from the other *Biomphalaria glabrata* populations. (2) The highly inbred laboratory stocks, 6-4-1 and 10R2, had the modal karyotype for the species. (3) *Biomphalaria tenagophila* also had the modal karyotype for *Biomphalaria glabrata.* Certainly if any karyotypic divergence were to occur, we would expect the differences either at the species level or in laboratory stocks that had been highly inbred and were, therefore, subject to random fixation of gene rearrangements. This evidence suggests that the Dominican Republic sample of *Biomphalaria glabrata* was fixed for chromosomal rearrangements not typical of the species as a whole, and that this fixation event is not a usual occurrence in other populations. Interestingly, genetic distances measured by Mulvey (1981) on the basis of 22 enzyme loci between *Biomphalaria glabrata*/Dominican Republic and other populations of *Biomphalaria glabrata* were within the range of full species of *Drosophila,* indicating that there may be something exceptional about that particular population.

In an absolute sense, *Bulinus* and *Biomphalaria* are both karyotypically conservative. Except for cases of polyploidy, chromosome numbers throughout the family Planorbidae are constant. This is in contrast to the case in land snails of the families Oreohelicidae and Camaenidae, in which Babrakzai *et al.* (1974) have found species differing in chromosome number (n = 26 to 32) without involvement of polyploidy. In an extreme case of chromosome number change, the Indian muntjac (Chordata: Mammalia) is distinguished from its morphologically almost identical congener, the Reeve's muntjac, with diploid chromosome numbers of $2n$ = 6 and $2n$ = 46, respectively (Wurster & Benirschke, 1970). It should, therefore, be stressed that in considering the differences in karyotype divergence among species of *Biomphalaria* as opposed to that among species of *Bulinus*, we are truly speaking only of a relative difference in karyotype conservatism.

The degree of karyotypic diversity observed among closely-related species of mollusks is not easily assessed, since only about 0.5% of all species have been karyotyped (Patterson & Burch, 1978). We know only a few groups in reasonable cytological detail, and those particular groups are not necessarily those in which other aspects of biology have been adequately studied. Four important reviews are available—Burch & Huber (1966) on polyploidy, Patterson (1969), which deals with all mollusks, Patterson (1973), dealing only with gastropods, and Patterson & Burch (1978), restricted in scope to the Pulmonata.

Sixty-one percent of the families of Pulmonata have been studied cytologically (Patterson & Burch, 1978). The haploid chromosome number varies from n = 5 to n = 72. Burch (1967b) notes, however, that chromosome numbers are quite constant within lower taxonomic categories, such as families.

Polyploidy is known in several snail genera (Burch & Huber, 1966), including *Melanoides* (Jacob, 1958), *Gyraulus* (Burch, 1960), *Ancylus* (Burch *et al.,* 1960), *Ferrissia* (Burch *et al.,* 1960) and *Bulinus* (Patterson & Burch, 1978). Seven percent of snails of the family Planorbidae, in which self-fertilization is a known possibility, are polyploids (Burch & Huber, 1966).

The best studied group from a karyotypic point of view is the genus *Semisulcospira,* found in Japan (Burch, 1967a; Patterson, 1969). Twelve species were karyotyped, having a diploid chromosome number ranging from n = 7 to n = 20. Burch (1967b) presented strong evidence for divergence in chromosome morphology as well as in chromosome number. It is possible that these chromosomal changes occurred by reciprocal translocations or by loss or addition of heterochromatin.

Reeder *et al.* (1975) and Reeder & Miller (1975) have produced evidence for changes in chromosome morphology in land snails of the genus *Ashmunella.* These appear to be minor changes in centromere position. Because work is in progress to develop G-banding techniques for these snails (Babrakzai *et al.,* 1975, and personal communication), the degree of karyotypic differentiation in this group may soon be known in great detail.

Breeding Biology, Chromosomal Divergence and Genetic Variation

We define the term "breeding biology," for the purposes of this study, to include all aspects of behavior, genetic system, ecology, and physiology which influence the distribution of genotype frequencies in future generations. This definition is intentionally broad, because it is important to consider the effects

of a variety of factors, such as inbreeding, selfing, and social structuring, on degrees of chromosomal divergence among taxa, and on levels of genetic variation within or among taxa.

It is now generally accepted that chromosomal evolution is more rapid in small or highly inbred populations than in large, outbreeding populations (White, 1973). Breeding biology and rate of karyotypic evolution may influence the mode of speciation in organisms, hence our approach to studying speciation and in recognizing species in various groups. White (1973, 1978) favors the view that speciation takes place in isolated populations. He believes that in a large isolated population little chromosomal change will occur, but that in small isolates, gene rearrangements may be fixed rapidly. The latter is termed the stasipatric mode of speciation (White, 1968). In some groups, such as cetacean mammals, little chromosomal differentiation has accompanied speciation.

Wilson *et al.* (1975) and Bush *et al.* (1977) have reviewed evidence for a connection between rapid chromosomal evolution and rapid speciation, both of which they assume are related to small effective population size. They found that in mammals both speciation rates and rates of karyotypic evolution are higher than among frogs and amphibians, and argued that this is due to a reduced effective population size owing to social structuring of mammalian populations.

Several studies seem to have established a connection between population structure and electrophoretically-detected genetic variation. Nevo (1978) summarized the evidence from a number of studies on electrophoretic variation in animals and concluded that the breeding system does, indeed, influence the observed patterns of genetic variation. Gottlieb (1980) reviewed the data on 49 plant species, and found significant differences between inbreeders and outbreeders with respect to heterozygosity and proportion of loci polymorphic, both measures being higher in the outbreeders. He did not find a significant difference in genetic identity among populations of outbreeders as opposed to inbreeders.

Levin (1975) directly tested the hypothesis that inbred species would have a lower proportion of polymorphic loci, a higher deficit of heterozygotes, and a greater between-population heterogeneity than a closely-related sympatric outbreeder. He found that *Phlox drummondii*, an obligate outcrosser, has a proportion of polymorphic loci, *P*, of 0.19, a heterozygote deficit of 0.43, while *P. pilosa,* which can and does self-fertilize to a significant degree, has a *P* of 0.11 and a heterozygote deficit of 0.67. *P. pilosa* was also characterized by a greater between-population heterogeneity of allele frequencies than was *P. drummondii.* Schoen (1982) reported similar findings in inbred versus outbred populations of one species, *Gilia achilleifolia.*

Evidence for Inbreeding in *Bulinus* and *Biomphalaria*

Several lines of evidence lead to the suggestion that *Bulinus* snails are more highly inbred than snails of the genus *Biomphalaria.* These lines of evidence include (1) the occurrence of polyploidy in *Bulinus*, which often presupposes at least temporary relief from sexual reproduction, (2) the high frequency of aphallic individuals in some populations of the *Bulinus truncatus/tropicus* complex, and the scarcity of aphallic specimens in *Biomphalaria*, (3) experimental evidence for the success of genetic crossing experiments in *Biomphalaria* and the frequent failure of such experiments in *Bulinus*, and (4) evidence of a greater degree of genic polymorphism and a lower degree of interpopulation and interspecific differentiation at the biochemical level in *Biomphalaria* as opposed to *Bulinus.*

White (1973, 1978) regards outcrossing as one of the most serious barriers to the establishment of polyploidy in animal species. The only example of polyploid animals with a continuously bisexual reproductive habit of which we are aware are the autotetraploid frogs (Ceratophrydidae) of South America (Becak *et al.,* 1966; Bogart & Tandy, 1973; see White, 1973). In all other cases, the animals either are or were facultatively self-fertilizing or parthenogenetic. It is reasonable, therefore, to postulate that in *Bulinus*, the origin of polyploidy was accompanied by a self-fertilization event which overcame the sterility one might have expected if the first polyploid individual had bred with a diploid organism.

The lack of a male copulatory organ (the aphallic condition) necessitates self-fertilizing or behavior as a female partner in facultatively-selfing hermaphroditic animals like *Bulinus* snails (Brown, 1980). Proportions of snails aphallic varying from 0 to 100% have

been reported in various populations of *Bulinus truncatus* (Brown, 1980). Only in a population with absolutely no euphallic individuals would self-fertilization be obligatory. Even in a population with an intermediate proportion of aphallic individuals, however, inbreeding may be significant, because many members of the population may in fact encounter only a small sampling of the local population for mating. We have no direct evidence of the frequency of self-fertilization. *Bulinus* has been observed copulating in the field (Brown, 1980), but no one has been able to demonstrate self-fertilization in the field. Self-fertilization has been demonstrated in isolation experiments in the laboratory (Goldman, unpubl., and observations in numerous laboratories). Nevertheless, the lowering of the proportion of individuals that can act as a male partner would be expected to result in a decrease in effective population size and an increase in inbreeding (Hartl, 1980), especially if the aphallic condition is shown to be heritable (D. L. Hartl, Washington University, personal communication).

The aphallic condition reaches appreciable frequencies only within the *Bulinus truncatus/tropicus* complex, and is encountered only rarely in other species groups of *Bulinus* and in the genus *Biomphalaria* (Brown, 1980). On these grounds, one might expect to see some reduction in outbreeding in *Bulinus* as compared to *Biomphalaria*.

C. S. Richards (Bethesda Research Institute, personal communication) has reported difficulty in crossing *Bulinus* snails for genetic experiments, but remarkable success in crossing *Biomphalaria* stocks under the same conditions. In fact, he reports some cases in which three *Biomphalaria* snails placed in a jar are known to have cross-fertilized in all possible combinations on the basis of genetic markers observed among their offspring. Paraense (1956) reported that *Biomphalaria* snails do outcross, and that a *Biomphalaria* population may be regarded as randomly mating.

Because of the enormous importance of *Bulinus* and *Biomphalaria* as potential vectors for human schistosomiasis, a great deal of work has been done on protein electrophoresis of *Bulinus* and *Biomphalaria*. Unfortunately, much of the data are of limited use in the present context because the (1) experiments were designed with other purposes in mind, (2) investigators have not always been able to confirm a genetic basis for the patterns seen and (3) many workers have used the digestive gland as a tissue source for electrophoretic assays. The digestive gland has been observed by some workers (see Wright *et al.,* 1966) to have a great deal of esterase activity, but the gel pattern varies with the physiological condition of the snail. It is possible that proteolytic enzymes in the digestive system alter the patterns, or that foreign material in the digestive tract interferes.

Recent work by Mulvey & Vrijenhoek (1981; Mulvey, 1981, and personal communication) has produced a good deal of critical data on *Biomphalaria*. In laboratory strains of *Biomphalaria glabrata,* more than 50% of the loci were polymorphic either within or among strains (Mulvey and Vrijenhoek, 1981). In field collections, representing a narrower portion of the species range than did the laboratory strains, only about 15% of loci were polymorphic (Mulvey, personal communication). The results suggested that local populations of *Biomphalaria glabrata* are outcrossing and randomly mating (Mulvey, 1981, and personal communication). There is some degree of differentiation between localities in *Biomphalaria glabrata* (Mulvey, 1981, and personal communication), consistent with differences found among local races or subspecies of *Drosophila.*

Narang *et al.* (1981) reported similar levels of polymorphism and heterozygosity within populations of *Biomphalaria glabrata* in Brazil. Their genetic distance values were slightly larger than those reported by Mulvey (1981). Although most populations fell within the expected range, some populations were as divergent as full species of *Drosophila.*

Results compatible with those of Mulvey & Vrijenhoek (1981), Mulvey (1981) and Narang *et al.* (1981) have been reported for other species of *Biomphalaria* by Wium-Andersen (1973) and Ukoli (1974) in less extensive surveys using single enzyme systems. The details of work by Henriksen & Jelnes (1980) using twelve enzyme systems have not yet been published. These data are consistent with our contention that *Biomphalaria* is an outbreeder.

Electrophoretic studies on *Bulinus* snails have generally not revealed the same level of variation found in *Biomphalaria.* The results cannot be accepted without question, however, since the published survey work with *Bulinus* has not been as extensive, frequently being based on just a few enzyme systems

(*e.g.* Wright & File, 1968; Jelnes, 1977) or on a small population sample (*e.g.* Wright & Rollinson, 1979).

Wright and File (1968) studied esterase polymorphism in the digestive gland of fifty populations of *Bulinus.* They found low variation within populations, but that populations differed in frequencies of esterase phenotypes. The variation between populations was greater than that between species.

In surveys involving the enzyme phosphoglucose isomerase (PGI), Jelnes (1977) found some evidence for outbreeding in *Bulinus tropicus* and *Bulinus permembranaceus* (Preston). In another survey of fifty populations of *Bulinus truncatus,* however, Jelnes (1978) found only two that had a variant electromorph of PGI, and that variant was fixed in both of those populations. He favored the hypothesis that reproduction is parthenogenetic in *Bulinus truncatus.* Jelnes (1978) mentions confirming evidence from esterase electrophoresis. For *Bulinus forskalii,* Jelnes (1980) concluded that both self-fertilization and cross-fertilization take place.

Wurzinger (1979) surveyed five populations of *Bulinus truncatus* and found no variation within any of them. Some were apparently fixed heterozygotes. Four of the populations he sampled were primarily aphallic, but the one estimated to be 100% euphallic also did not show variation. Wurzinger & Saliba (1979), however, showed segregation for acid phosphatase alleles in a Syrian population of *Bulinus truncatus.*

On the basis of five enzyme systems studied in digestive gland extracts, Wright & Rollinson (1981) concluded that there is outbreeding in *Bulinus permembranaceus,* but that parthenogenesis or selfing must also occur.

The evidence for a genetic polymorphism for isozymes within populations of *Biomphalaria* seems compelling, and the available data suggest that outbreeding is important. Paraense (1956) stated that populations of Planorbidae should be considered outbreeding on the basis of his studies of *Biomphalaria.*

Many authors have concluded that genetic variation within *Bulinus* populations is slight or non-existent, as one would expect of a highly inbred population or of one in which parthenogenetic reproduction was frequent. Further, most authors have found that a sufficient number of enzyme characters does allow one to distinguish between populations, suggesting that populations diverge substantially in electromorph frequencies. This, too, is as one would expect under a hypothesis of inbreeding.

The evidence outlined in this section is consistent with the hypothesis that *Bulinus* snails are more highly inbred than are *Biomphalaria* snails. As snails of both genera could self, this is not a strict dichotomy; one must consider a continuum between inbreeders and outbreeders. Further, individual populations or species within these genera may differ tremendously in their breeding habits (Wright & Rollinson, 1979), as evidenced by the greatly varying frequency (0 to 100%) of aphallic individuals found in *Bulinus truncatus.*

CONCLUSION

In this report, we have presented data and reviewed data from the literature which indicate a greater degree of karyotypic divergence among species of *Bulinus* than among species of *Biomphalaria,* and have adduced evidence to show that *Bulinus* is more subject in inbreeding than is *Biomphalaria.* We now submit as a working hypothesis that there is a relationship, causal or otherwise, between the reduced inbreeding and the reduced degree of karyotypic divergence observed among *Biomphalaria* as opposed to *Bulinus* species.

If borne out by further studies, the relationship between inbreeding and an increased rate of karyotypic divergence in planorbid snails would be consistent with the hypothesis of Wilson *et al.* (1975) and Bush *et al.* (1977) that small effective population size and rapid karyotypic evolution are associated and may be causally related. Firm establishment of the connection for *Bulinus* and *Biomphalaria* clearly requires more work. The summary of the evidence presented points future workers toward the deficiencies in the available data.

Perhaps the easiest methods of further investigating this problem in *Bulinus* and *Biomphalaria* would be cytogenetic and electrophoretic techniques. Good results from a number of banding techniques, including C-banding, are important in a thorough analysis of karyotypic evolution (Hsu, 1981), for the morphology and size of metaphase chromosomes reveal only a fraction of the total number of rearrangements that may have occurred. The banding studies must then be

extended to (1) *Biomphalaria* species from the Old World as well as from the New, (2) at least two populations of each species of *Bulinus,* so that we may get a better idea of the differences between stocks, and (3) two critical populations which are discussed below.

Brown (1976) reported populations of diploid and tetraploid *Bulinus* living sympatrically in the highlands of Kenya in which the diploids appear electrophoretically more similar to the polyploids than to other diploid species groups some distance outside of the region. This may be evidence of a recent origin of the polyploids from the sympatric diploids. It may be interesting to assess the degree of karyotypic divergence between these two sympatric species, and to compare this with the sort of karyotypic divergence that has occurred between species which we do not believe to have arisen so recently.

In addition to obtaining more detailed cytogenetic information on a number of interesting populations, it will be necessary to do electrophoretic studies similar in extent and rigor to those of Mulvey & Vrijenhoek (1981; Mulvey, 1981). One should obtain estimates of the fraction of loci polymorphic and of the fraction of individuals heterozygous in each defined population, and over the species as a whole. The deficit of heterozygotes as compared to Hardy-Weinberg expectations should be determined, and inbreeding statistics should be calculated. Various indices of genetic similarity and distance should be calculated to assess the degree of differentiation among various populations of a species, and among various species within genera. When this information is obtained for the taxa in which we are interested, we will be able to verify or discredit some of the predictions of the "inbreeding hypothesis." (1) Genetic variation within a local population should be lower within an inbred than within an outbred population. (2) Heterozygote frequency should be lower than expected in an inbred population; one might expect a greater deficit of heterozygotes, or a greater homozygote excess, in the more inbred population. (3) Genetic differentiation among populations of an inbred group should be greater than genetic differentiation among populations of a less inbred group, provided that other factors, such as selection and migration rates, do not confound the issue.

Wilson *et al.* (1975) and Bush *et al.* (1977) have reviewed a great deal of data concerning rates of mammalian and amphibian karyotypic evolution in relation to rates of speciation. There are, however, two important limitations we wish to stress. First, these authors define karyotypic change as a change in chromosome number or in chromosome arm number (fundamental number). This ignores many changes in karyotype, such as inversions, translocations, and heterochromatin deletions, which could result in substantial and biologically significant karyotypic change in the absence of a numerical change in the chromosomes. Kornfield *et al.* (1979) have shown that two species of cichlid fishes that appeared to be karyotypically identical on the basis of standard chromosome techniques differed in C-banding patterns. Second, the comparison of mammals and amphibians is too "coarse" to provide a test of a hypothesis with predictive value. There are karyotypically conservative groups of mammals (bats; Baker & Bickham, 1980), and there are karyotypically variable amphibian groups (ceratophrydid frogs; Becak *et al.,* 1966). Baker & Bickham (1980) observed contrasting degrees of karyotypic evolution in various groups of bats, and suggested that even single genera cannot be characterized as karyotypically conservative or non-conservative; species may differ widely in rate of karyotypic evolution as well as in other aspects of biology. A species cannot be assumed to have great potential for karyotypic change simply because it belongs to an appropriate genus; the genus gets its tendency for rapid karyotypic change because of an "average" condition of species-richness or small effective population size. An individual species should have the predicted attribute, *e.g.* rapid karyotypic evolution, only if it also has the predicting attribute, *e.g.* small effective population size.

Confirming data for the hypothesized relationships among breeding biology, speciation rates, and rates of karyotypic evolution, must be obtained through the kind of work described here and in Baker & Bickham (1980). These studies compare closely-related taxa so as to minimize confounding biological differences, and consider minor karyotypic rearrangements revealed by chromosome banding techniques as well as gross karyotypic changes in chromosome or fundamental number.

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REFERENCES CITED

ANSARI, N., 1973, *Epidemiology and control of schistosomiasis (bilharziasis).* University Park Press, Baltimore, 752 p.

BABRAKZAI, N., MILLER, W. B. & WARD, O. G., 1974, Cytotaxonomy of some Arizona Oreohelicidae (Gastropoda: Pulmonata). *Bulletin of the American Malacological Union,* 40: 4–11.

BABRAKZAI, N., WARD, O. G. & MILLER, W. B., 1975, The introduction of Giemsa and centromeric banding techniques of chromosomes to molluscan cytotaxonomy. *Bulletin of the American Malacological Union,* 41: 67.

BAKER, R.J. & BICKHAM, J.W., 1980, Karyotypic evolution in bats: evidence of extensive and conservative chromosomal evolution in closely related taxa. *Systematic Zoology,* 29: 239–253.

BECAK, M. L., BECAK, W. & RABELLO, M. N., 1966, Cytological evidence of constant tetraploidy in the bisexual South American frog *Odontophrynus americanus. Chromosoma,* 19: 188–193.

BOGART, J. P. & TANDY, M., 1973, Polyploid amphibians: three more diploid-tetraploid species of frogs. *Science,* 193: 334–335.

BROWN, D. S., 1976, A tetraploid freshwater snail (Planorbidae: *Bulinus*) in the highlands of Kenya. *Journal of Natural History,* 10: 257–267.

BROWN, D. S., 1980, *Freshwater snails of Africa and their medical importance.* Taylor and Francis, London, 487 p.

BURCH, J. B., 1960, Chromosomes of *Gyraulus circumstratius,* a freshwater snail. *Nature,* 186: 497–498.

BURCH, J. B., 1967a, Chromosomes of intermediate hosts of human bilharziasis. *Malacologia,* 5: 127–135.

BURCH, J. B., 1967b, Chromosomes and systematics of molluscs. *Annual Reports of the Papua and New Guinea Scientific Society,* 18: 26–28.

BURCH, J. B., BASCH, P. F. & BUSH, L. L., 1960, Chromosome numbers in ancylid snails. *Revista Portuguesa de Zoologia e Biologia Geral,* 2: 199–204.

BURCH, J. B. & HUBER, J. M., 1966, Polyploidy in mollusks. *Malacologia,* 5: 41–43.

BUSH, G. L., CASE, S. M., WILSON, A. C. & PATTON, J. L., 1977, Rapid speciation and chromosomal evolution in mammals. *Proceedings of the National Academy of Science,* 74: 3942–3946.

CLAUGHER, D., 1971, Karyotype analysis of bulinid snails. *Bulletin of the World Health Organization (Geneva),* 45: 855–858.

GIACOMOZZI, R. O., RIVA, R. & VIDA, O. R., 1979, The chromosomes of *Biomphalaria tenagophila* (Gastropoda—Basommatophora—Planorbidae). *Cytologia,* 44: 385–389.

GILL, J. J. B. & CAIN, A. J., 1980, The karyotype of *Cepaea sylvatica* (Pulmonata: Helicidae) and its relationship to those of *C. hortensis* and *C. nemoralis. Biological Journal of the Linnean Society,* 14: 293–301.

GOLDMAN, M. A., 1981, Polyploidy and evolution in the genus *Bulinus* (Mollusca: Planorbidae). Ph.D. thesis, Purdue University, West Lafayette, Indiana, 110 p.

GOLDMAN, M. A., LoVERDE, P. T. & CHRISMAN, C. L., 1980, Comparative karyology of the freshwater snails *Bulinus tropicus* and *B. natalensis* (Mollusca: Planorbidae). *Canadian Journal of Genetics and Cytology,* 22: 361–367.

GOLDMAN, M. A., LoVERDE, P. T. & CHRISMAN, C. L., 1983a, Hybrid origin of polyploidy in freshwater snails of the genus *Bulinus* (Mollusca: Planorbidae). *Evolution,* 37: 592–600.

GOLDMAN, M. A., LoVERDE, P. T., CHRISMAN, C. L., FRANKLIN, D. A., MATTHEWS, F., PITCHFORD, R. J. & RICHARDS, C. S., 1983b, Nucleolar organizer regions in *Biomphalaria* and *Bulinus* snails. *Experientia,* 39: 911–913.

GOTTLIEB, L. D., 1980, Electrophoretic evidence and plant populations. *Progress in Phytochemistry,* 7: 1–46.

HARTL, D. L., 1980, *Principles of population genetics.* Sinauer Associates, Sunderland, Massachusetts, 488 p.

HENRIKSEN, U. B. & JELNES, J. E., 1980, Experimental taxonomy of *Biomphalaria* (Gastropoda: Planorbidae). I. Methods for experimental taxonomic studies on *Biomphalaria* carried out by horizontal starch gel electrophoresis and staining of twelve enzymes. *Journal of Chromatography,* 188: 169–176.

HOWELL, W. M. & BLACK, D. A., 1980, Controlled silver-staining of nucleolus organizer regions with a protective coloidal developer: a 1-step method. *Experientia,* 36: 1014.

HSU, T. C., 1981, Cytogenetics: today and tomorrow, p. 29–46 *in* ARRIGHI, F. E., RAO, P. N. & STUBBLEFIELD, E. eds., *Genes, chromosomes, and neoplasia.* Raven Press, New York.

JACOB, J., 1958, Cytological studies of Melaniidae (Mollusca) with special reference to parthenogenesis and polyploidy. II. A study of meiosis in the rare males of the polyploid race of *Melanoides tuberculatus* and *M. lineatus. Transactions of the Royal Society, Edinburgh,* 63: 433–445.

JELNES, J. E., 1977, An electrophoretic character

useful in the distinction between *Bulinus tropicus* and *B. permembranaceus* (Gastropoda, Planorbidaea). *Steenstrupia,* 4: 139–141.

JELNES, J. E., 1978, Experimental taxonomy of *Bulinus* (Gastropoda: Planorbidae). I. Electrophoretic studies on esterase and phosphoglucose isomerase of *Bulinus truncatus.* Organ distribution, geographical variation and taxonomic implications. *Archiv für Molluskenkunde,* 109: 237–248.

JELNES, J. E., 1980, Experimental taxonomy of *Bulinus* (Gastropoda: Planorbidae). III. Electrophoretic observations on *Bulinus forskalii, B. browni, B. barthi,* and *B. scalaris* from East Africa, with additional electrophoresis data on the subgenus *Bulinus sensu stricto* from other parts of Africa. *Steenstrupia,* 6: 177–193.

KORNFIELD, I. L., RITTE, U., RICHLER, C. & WAHRMAN, J., 1979, Biochemical and cytological differentiation among cichlid fishes of the Sea of Galilee. *Evolution,* 33: 1–14.

LEVAN, A., FREDGA, K. & SANDBERG, A. A., 1964, Nomenclature for centromeric position on chromosomes. *Hereditas,* 52: 201–220.

LEVIN, D. A., 1975, Interspecific hybridization, heterozygosity and gene exchange in *Phlox. Evolution,* 29: 37–51.

MULVEY, M., 1981, Genetics and population biology of the snail *Biomphalaria* (Gastropoda: Planorbidae). Ph.D. thesis, Rutgers University, New Brunswick, New Jersey.

MULVEY, M. & VRIJENHOEK, R. C., 1981, Genetic variation among laboratory strains of the planorbid snail *Biomphalaria glabrata. Biochemical Genetics,* 19: 1169–1182.

NARANG, N., 1974, Cytogenetic efforts of radiation on the planorbid snail, *Biomphalaria glabrata. Caryologia,* 27: 385–393.

NARANG, N., NARANG, S., MONTEIRO, W. & ALMEIDA, J. M. C. DE, 1981, Genetic differentiation between populations of *Biomphalaria glabrata* (Mollusca: Pulmonata) located in the state of Bahia and in the state of Minas Gerais (Brazil). *Brazilian Journal of Genetics,* 4: 269–279.

NEVO, E., 1978, Genetic variation in natural populations. Patterns and theory. *Theoretical and Population Biology,* 13: 121–177.

PAGE, C., 1978, The karyotype of the land snail *Cepaea nemoralis* (L.). *Heredity,* 41: 321–325.

PARAENSE, W. L., 1956, A genetic approach to the systematics of planorbid molluscs. *Evolution,* 10: 403–407.

PARIS CONFERENCE, 1971, Standardization in human cytogenetics. *Cytogenetics,* 11: 313–362.

PATTERSON, C. M., 1969, Chromosomes of mollusks, p. 635–686 *in Marine Biological Association of India, Proceedings of Symposium on Mollusca,* Part II.

PATTERSON, C. M., 1971, A karyotype technique using freshwater snail embryos. *Malacological Review,* 4: 27.

PATTERSON, C. M., 1973, Cytogenetics of gastropod mollusks. *Bulletin of the American Malacological Union,* 39: 34–35.

PATTERSON, C. M. & BURCH, J. B., 1978, Chromosomes of pulmonate molluscs, p. 171–217 *in* FRETTER, V. & PEAKE, J. eds., *Pulmonates: systematics, evolution and ecology,* Vol. 2A. Academic Press, New York.

RAGHUNATHAN, L., 1976, The karyotype of *Biomphalaria glabrata,* the snail vector of *Schistosoma mansoni. Malacologia,* 15: 447–450.

REEDER, R. L. & MILLER, W. B., 1975, Karyotype studies in *Ashmunella* (Pulmonata: Polygyridae). *Bulletin of the American Malacological Union,* 41: 71.

REEDER, R. L., BABRAKZAI, N. & MILLER, W. B., 1975, Cytotaxonomic studies in Polygyridae (Gastropoda: Pulmonata). *Journal of the Arizona Academy of Sciences, Proceedings* Supplement, p. 15.

SCHOEN, D. J., 1982, Genetic variation and the breeding system of *Gilia achilleifolia. Evolution,* 36: 361–370.

SCHUTTE, C. H. J., 1966, Observations on two South African bulinid species of the *truncatus* group (Gastropoda, Planorbidae). *Annals of Tropical Medicine and Parasitology,* 60: 106–113.

UKOLI, F. M. A., 1974, Electrophoretic studies on foot muscle esterases of some African *Biomphalaria* species. *Malacological Review,* 7: 15–24.

WHITE, M. J. D., 1968, Modes of speciation. *Science,* 159: 1065–1068.

WHITE, M. J. D., 1973, *Animal cytology and evolution.* Cambridge University Press, Cambridge, England, 961 p.

WHITE, M. J. D., 1978, *Modes of speciation.* Freeman, San Francisco, 455 p.

WILSON, A. C., BUSH, G. L., CASE, S. M. & KING, M. C., 1975, Social structuring of mammalian populations and rate of chromosomal evolution. *Proceedings of the National Academy of Sciences,* 72: 5061–5065.

WIUM-ANDERSEN, G., 1973, Electrophoretic studies on esterases of some African *Biomphalaria* spp. (Planorbidae). *Malacologia,* 12: 115–122.

WRIGHT, C. A. & FILE, S. K., 1968, Digestive gland esterases in the genus *Bulinus* (Mollusca, Planorbidae). *Comparative Biochemistry and Physiology,* 27: 871–874.

WRIGHT, C. A. & ROLLINSON, D., 1979, Analysis of enzymes in the *Bulinus africanus* group (Mollusca: Planorbidae) by isoelectric focusing. *Journal of Natural History,* 13: 263–273.

WRIGHT, C. A. & ROLLINSON, D., 1981, Analysis of enzymes in the *Bulinus tropicus/truncatus* complex (Mollusca: Planorbidae). *Journal of Natural History,* 15: 873–885.

WRIGHT, C. A., FILE, S. K. & ROSS, G. C., 1966, Studies on the enzyme systems of planorbid snails. *Annals of Tropical Medicine and Parasitology,* 60: 522–525.

WU, S. K., 1972, Comparative studies on the poly-

ploid series of the African snail genus *Bulinus* (Basommatophora: Planorbidae). *Malacological Review,* 5: 95–164.

WURSTER, D. H. & BENIRSCHKE, K., 1970, Indian muntjac, *Muntiacus muntiak:* a deer with a low diploid chromosome number. *Science,* 168: 1364–1366.

WURZINGER, K.-H., 1979, Allozymes of Ethiopian *Bulinus sericinus* and Egyptian *Bulinus truncatus. Malacological Review,* 12: 51–58.

WURZINGER, K.-H. & SALIBA, E. K., 1979, A cytological and electrophoretic comparison of Jordanian *Bulinus* with three other tetraploid *Bulinus* populations. *Malacological Review,* 12: 59–65.

APPENDIX 1. Relative chromosome lengths and arm ratios for *Biomphalaria glabrata* NIH 6-4-1.[1]

Group no.	No. of pairs	Relative length[2] (x ± SE)	Arm ratio[3] (x ± SE)
I	1	10.71 ± 0.202	0.905 ± 0.013
II	2	6.74 ± 0.131	0.865 ± 0.014
III	3	6.59 ± 0.133	0.694 ± 0.017
IV	1	6.10 ± 0.119	0.191 ± 0.015
V	3	5.31 ± 0.125	0.836 ± 0.012
VI	1	4.03 ± 0.098	0.441 ± 0.034
VII	4	4.94 ± 0.094	0.715 ± 0.020
VIII	1	3.86 ± 0.094	0.466 ± 0.027
IX	2	3.69 ± 0.077	0.734 ± 0.041

[1]Based on measurements of 8 karyotyped cells.
[2]Expressed as percent total complement length (Paris Conference, 1971).
[3]Expressed as length of short arm/length of long arm (Paris Conference, 1971).

APPENDIX 2. Relative chromosome lengths and arm ratios for *Biomphalaria glabrata* NIH 10R2.[1]

Group no.	No. of pairs	Relative length[2] (x ± SE)	Arm ratio[3] (x ± SE)
I	1	10.34 ± 0.195	0.898 ± 0.018
II	2	6.97 ± 0.118	0.864 ± 0.020
III	3	6.36 ± 0.124	0.710 ± 0.018
IV	1	6.31 ± 0.115	0.171 ± 0.015
V	3	5.21 ± 0.111	0.866 ± 0.014
VI	1	4.13 ± 0.132	0.436 ± 0.055
VII	4	5.14 ± 0.071	0.694 ± 0.026
VIII	1	4.39 ± 0.142	0.561 ± 0.036
IX	2	3.55 ± 0.078	0.739 ± 0.023

[1]Based on measurements of 10 karyotyped cells.
[2]Expressed as percent total complement length (Paris Conference, 1971).
[3]Expressed as length of short arm/length of long arm (Paris Conference, 1971).

APPENDIX 3. Relative chromosome lengths and arm ratios for *Biomphalaria glabrata* from Dominican Republic.[1]

Group no.	No. of pairs	Relative length[2] (x ± SE)	Arm ratio[3] (x ± SE)
I	1	10.53 ± 0.284	0.887 ± 0.019
II	2	6.80 ± 0.117	0.823 ± 0.028
III	1	7.69 ± 0.263	0.696 ± 0.032
IV	1	6.39 ± 0.061	0.168 ± 0.011
V	4	5.80 ± 0.094	0.677 ± 0.025
VI	1	4.23 ± 0.204	0.412 ± 0.055
VII	3	5.20 ± 0.106	0.896 ± 0.010
VIII	2	4.36 ± 0.090	0.460 ± 0.030
IX	3	3.26 ± 0.121	0.810 ± 0.024

[1]Based on measurements of 9 karyotyped cells.
[2]Expressed as percent total complement length (Paris Conference, 1971).
[3]Expressed as length of short arm/length of long arm (Paris Conference, 1971).

APPENDIX 4. Relative chromosome lengths and arm ratios for *Biomphalaria glabrata* from Brazil.[1]

Group no.	No. of pairs	Relative length[2] (x ± SE)	Arm ratio[3] (x ± SE)
I	1	10.62 ± 0.209	0.855 ± 0.025
II	2	6.82 ± 0.141	0.866 ± 0.015
III	3	6.94 ± 0.159	0.603 ± 0.031
IV	1	6.61 ± 0.088	0.156 ± 0.016
V	3	5.00 ± 0.092	0.861 ± 0.014
VI	1	3.58 ± 0.188	0.321 ± 0.038
VII	4	5.29 ± 0.090	0.692 ± 0.019
VIII	1	4.18 ± 0.104	0.537 ± 0.041
IX	2	3.61 ± 0.117	0.794 ± 0.034

[1]Based on measurements of 8 karyotyped cells.
[2]Expressed as percent total complement length (Paris Conference, 1971).
[3]Expressed as length of short arm/length of long arm (Paris Conference, 1971).

APPENDIX 5. Relative chromosome lengths and arm ratios for *Biomphalaria straminea*.[1]

Group no.	No. of pairs	Relative length[2] (x ± SE)	Arm ratio[3] (x ± SE)
I	1	9.08 ± 0.245	0.923 ± 0.037
II	2	6.06 ± 0.062	0.912 ± 0.034
III	1	6.72 ± 0.333	0.716 ± 0.046
IV	1	5.94 ± 0.128	0.264 ± 0.015
V	4	5.28 ± 0.130	0.739 ± 0.033
VI	1	3.88 ± 0.107	0.343 ± 0.080
VII	5	5.47 ± 0.141	0.879 ± 0.037
VIII	1	4.93 ± 0.199	0.624 ± 0.036
IX	2	4.47 ± 0.182	0.700 ± 0.072

[1]Based on measurements of 2 karyotyped cells.
[2]Expressed as percent total complement length (Paris Conference, 1971).
[3]Expressed as length of short arm/length of long arm (Paris Conference, 1971).

APPENDIX 6. Relative chromosome lengths and arm ratios for *Bulinus* sp. $2n = 36$ from Mazoe Dam.[1]

Group no.	No. of pairs	Relative length[2] (x ± SE)	Arm ratio[3] (x ± SE)
I	1	9.27 ± 0.302	0.921 ± 0.000
II	1	6.99 ± 0.176	0.727 ± 0.029
III	1	7.06 ± 0.182	0.854 ± 0.022
IV	2	6.32 ± 0.083	0.826 ± 0.018
V	2	5.81 ± 0.143	0.579 ± 0.041
VI	1	5.02 ± 0.362	0.349 ± 0.046
VII	4	5.41 ± 0.103	0.668 ± 0.036
VIII	2	5.04 ± 0.168	0.843 ± 0.024
IX	3	4.40 ± 0.106	0.487 ± 0.022
X	1	3.67 ± 0.231	0.643 ± 0.037

[1]Based on measurements of 7 karyotyped cells.
[2]Expressed as percent total complement length (Paris Conference, 1971).
[3]Expressed as length of short arm/length of long arm (Paris Conference, 1971).

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POPULATION STUDIES ON *BULINUS CERNICUS* FROM MAURITIUS

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ABSTRACT

Bulinus cernicus occurs commonly in a variety of habitats in the low-lying areas of Mauritius, where it serves as the intermediate host for the trematode *Schistosoma haematobium*. The morphological diversity within the group is such that recent investigators have suggested that more than one species of *Bulinus* may be present on the island. The present study was undertaken to gain a better understanding of the variation and population structure of this planorbid. Snails have been examined from 25 of the known habitats; live material was collected in 1979 and 1980. Preliminary observations on water chemistry at the different sites suggested that the finding of decollate shells was correlated with low levels of calcium. Quite striking differences in the shells and radular teeth occurred between populations; some of the differences were shown to persist in F_1 generation snails reared in the laboratory. Thirteen of the samples tested proved susceptible to a local strain of *S. haematobium* and snails were shown to be susceptible to *S. intercalatum* (Cameroun and Gabon), *S. bovis* (Kenya) and *S. haematobium* (Sudan and Kenya). Electrophoresis of six enzyme loci (MDH, PGM, GPI, AcP-A, AcP-B, HBDH) showed regional differences in gene frequencies. The mean heterozygosity per locus ranged from H = 0 to 0.265, with an overall mean of 0.09. Five of the 22 alleles identified were restricted to particular populations suggesting limited gene flow between habitats. No deviations were observed from Hardy-Weinberg expectations and isolated wild-caught snails produced offspring resulting from cross-fertilisation. Although isolated snails would self-fertilise, investigations utilising enzyme markers showed a marked preference for cross-fertilisation. Some crosses between snails from different samples were more successful than others. However, fertile F_1 snails were produced from a cross between individuals from the N.W. and S.E., differences in snails being greatest from these two areas. *B. cernicus* was shown to be capable of storing sperm for up to 70 days. The breeding biology is discussed in relation to the known variability and distribution. The results contrast markedly with the variation previously described in closely related species.

INTRODUCTION

In a recent synopsis of the freshwater genus *Bulinus*, Brown (1980) recognised 37 species which are commonly divided into four species groups. *B. cernicus* (Morelet, 1868) forms one of the nine species within the *B. forskali* group, which are characterised by their slender shells with the spires distinctly higher than the apertures when fully grown and by having the normal diploid chromosome number (2n = 36). The range of the *forskali* group comprises much of the African continent and includes some islands in the Western Indian Ocean (Fig. 1); *B. bavayi* (Dautzenberg, 1894) occurs in Madagascar and Aldabra, *B. forskali* (Ehrenberg, 1831) in Madagascar and *B. cernicus* in Mauritius. The genus is of particular medical and economic importance as many representatives serve as intermediate hosts for blood flukes of the

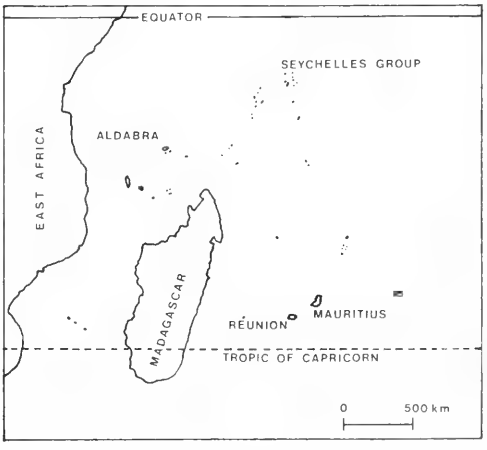

FIG. 1. Map of the S.W. Indian Ocean. *B. bavayi* is found on Madagascar and Aldabra, *B. forskali* on Madagascar and *B. cernicus* on Mauritius.

*Dr. C. A. Wright died on 19 June, 1983.

genus *Schistosoma,* parasites of man and domestic animals. *B. cernicus* is the sole representative of the genus in Mauritius where it acts as the intermediate host of *S. haematobium,* the bladder parasite of man.

For a long time *B. cernicus* was considered to be no more than an isolated race of the pan-African *B. forskali.* Mandahl-Barth (1957) remarked on the radular teeth of *B. cernicus* as the only difference in anatomy to distinguish the snails from *B. forskali. B. cernicus* has much narrower central and lateral teeth, the cusps being larger and the mesocone incompletely separated from the endocone. Wright (1971), however, drew attention to this being true of some populations but not of others. The variation in the shell was also remarked upon by Wright who reported that the spire is decollate in some populations.

The most recent survey of the distribution of *B. cernicus* on Mauritius is that of Courtois & Gébert (1979) although it was reported that this did not differ greatly from that of Cowper in 1953. Sites for the most part were clustered in the N.W. and S.E. low lying parts of the island. Cowper (1953) noted that numbers of habitats had declined since the early 1930s, but Courtois & Gébert (1979) observed that the number of known sites had increased considerably since Cowper's survey and commented on the diverse nature of the habitats, i.e. muddy pools, slow running water and springs, concrete irrigation canals, sugar cane fields, watercress beds and coastal sandy habitats containing brackish water. They suggested that more than one species of *Bulinus* might be present, a possibility also mentioned by Cowper (1953). Snails collected from certain localities were found to differ markedly from specimens collected elsewhere with regard to the radulae, genital organs and electrophoretic results although details of these differences were not given.

Due in part to the application of electrophoretic methods there has been an increasing awareness of the genetic variation within *Bulinus* populations and an increasing use of enzymes as taxonomic characters (e.g. Jelnes, 1977, 1978; Wright & Rollinson, 1979, 1981; Rollinson and Southgate, 1979, Biocca *et al.,* 1979), although little attention has yet focussed on the members of the *B. forskali* group. Jelnes (1979) named two new species of *Bulinus* within the *forskali* group utilising α-glycerophosphate dehydrogenase alleles as the sole distinguishing characters, a decision that did not meet with total approval (Rollinson, 1979). Further data were provided by Jelnes (1980) for six species of the *forskali* group; he recorded very low amounts of genetic variation in wild populations of East African origin and it was inferred that snails within this group reproduced by both self-fertilisation and cross-fertilisation. He also mentioned the possibility of two closely related taxa occurring in Mauritius but unfortunately the enzyme data were inconclusive and based on old laboratory stocks. Further indications of differences between populations were provided by Frandsen (1979) who observed greater differences in infection rates between two samples of *Bulinus cernicus* exposed to *S. intercalatum* from Cameroun than those observed between populations of *B. forskali* collected from various parts of Africa.

Lack of enzyme variation in populations of *B. senegalensis* (Müller, 1781) in Senegambia was noticed by Wright, Rollinson & Goll (1979). It had been initially assumed that the isolated nature of the habitats favoured by *B. senegalensis* would provide a barrier to gene flow and that populations might well show enzymatic differences. The lack of variation was further emphasized by the recent finding of *B. senegalensis* in Northern Nigeria (Betterton, Fryer & Wright, 1983). These populations again proved invariant and identical to populations from Senegambia in the five enzymes tested.

The present study provided an additional opportunity to look at populations of a species, within the *forskali* group, with a limited distribution, in greater detail. This paper presents preliminary results utilising snails collected in Mauritius during 1979 and 1980. An attempt has been made to provide further information on both the morphological and enzymatic variation occurring within and between populations of *B. cernicus* and to see whether differences are correlated with susceptibility to infection with *Schistosoma* spp. Laboratory crosses have been performed to establish whether barriers exist to successful outcrossing and to gain further insight into the breeding strategies of these interesting hermaphrodites.

MATERIALS AND METHODS

Snails were collected in June–September 1979 (by the Cambridge Schistosomiasis Survey) and May–June 1980 (by Rollinson) with the help of the staff of the Department of

Medical Entomology, Mauritius. A thorough search was made of the known habitats and, when present, an attempt was made to collect snails at different places around the water body. When pools proved to be dry, a search was made for aestivating snails under stones and in the upper surface layers. Snails were returned to London where some were used to establish laboratory populations while others were sacrificed for morphological and enzyme analyses. All wild-caught snails were screened for the shedding of cercariae. Scanning electron microscope (SEM) studies of radulae were made with a Cambridge Instrument CoS 180. Enzyme analyses were made of malate dehydrogenase (MDH), phosphoglucomutase (PGM), glucose phosphate isomerase (GPI), acid phosphatase (AcP) and hydroxybutyrate dehydrogenase (HBDH) by isoelectric focusing following the methods of Wright & Rollinson (1979). Water samples (20 ml) were collected from all habitats and analysed by a SMA 1260 microautoanalyser for calcium following the method of Gitelman (1967). Certain populations, as detailed in the Results section, were tested for susceptibility to *Schistosoma haematobium* (Mauritius, Sudan and Kenya), *S. intercalatum* (Cameroun and Gabon) and to *S. bovis* (Kenya). Snails were exposed in groups of three in small pots to fifteen miracidia. Thirty to forty days later, snails were tested for cercarial shed; alternatively, snails which had been exposed to miracidia were sacrificed about twenty-five days later and the digestive glands were utilised for MDH, GPI and AcP analyses.

Details of the crossing and isolation experiments are to be found in the Results sections. Snails used in breeding experiments were isolated as hatchlings or young snails less than 3 mm high.

RESULTS

The localities from which *B. cernicus* has been collected for this study are listed in Table 1, along with the number of snails collected, habitat details and map references; the approximate location of the collecting sites (1–25) is shown in Fig. 2. The results from collections made in 1979 and 1980 have been amalgamated. The number of snails collected at the sites varied from 2 to 581, the largest number being found aestivating at site 12. The only districts of Mauritius not represented, but in which snails have been previously found, were Black River and Plains Wilhelms in the west of the island. All of the eight known habitats in these areas were negative in 1980. Three of the sites (4, 22, 23) from which *B. cernicus* had been obtained in 1979 were negative in 1980. The 1980 survey, however, was more extensive, with snails being collected at 21 sites compared to 13 in 1979. Other snails commonly found in association with *B. cernicus* included *Lymnaea mauritiana, Melanoides tuberculata* and *Physa borbonica.* Other molluscs encountered included *Bellamya bengalensis, Clithon longispina, Thiara* sp., *Neritina* sp., *Neritilia* sp. and *Gyraulus mauritianus.*

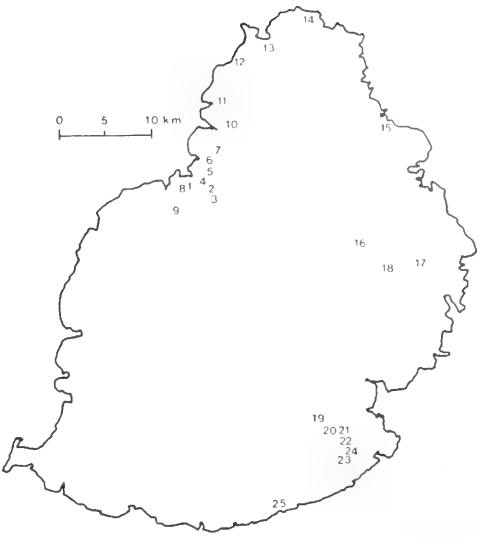

FIG. 2. Map of Mauritius showing sample localities.

Water chemistry

In habitats in which *B. cernicus* was found, the concentration of Ca^{++} varied from 0.31 m. mol./litre to 1.95 m. mol./litre. The values recorded for each habitat are given in Table 1. The Ca^{++} levels at sites where *B. cernicus* was not found fell within the same range. Undiluted water used for the maintenance of snails in the laboratory was 2.45 m. mol./litre. Values for levels of Na^{+}, K^{+} and Cl^{-} were very low and no appreciable variation was noticed between the habitats.

Shell

The shell is high spired but a characteristic of snails from some localities is the loss of the

TABLE 1. Details of localities for samples of *B. cernicus*.

Sample no.	Locality	Survivors (1979 & 1980)	Grid reference[1]	Description[2]	Ca^{++} m.mol/l	BM(NH) ref. no.
Port Louis						
1	Canal d'Etang	4	518706	cdc	—	727
2	Chateau d'Eau	23	532695	sp	0.93	815
3	Dauguet	115	535685	p	0.59	834
4	Pouce Stream	49	523706	cdc	—	728, 770
5	Vallée Pitot	101	528712	cdc	1.25	813
6	La Paix Stream	62	525713	cdc	0.69	726, 767, 818
7	Cité la Cure	91	535725	p/m	0.7	744, 814
8	Canal Caudan	36	512705	cdc	0.31	765, 833
Moka						
9	Pailles	47	490675	s/m	0.33	835
Pamplemousses						
10	Arsenal	2	557769	sp	1.95	832
11	Balaclava	57	542792	sp	0.90	823
12	Trou aux Biches	581	562840	p	0.66	725, 821
Rivière du Rempart						
13	Grande Baie	50	622882	p	0.95	829
14	Cap Malheureux	227	642901	p/m	0.65	729, 830
15	Roches Noires	96	755771	p	0.35	831
Flacq						
16	FUEL	113	722652	s	0.41	766, 820
17	Bel Air R. Sèche	24	780598	s	0.57	768, 836
18	Clémencia	101	743596	wcb	0.40	769, 822
Grand Port						
19	Mare d'Albert	156	657418	p	0.51	819
20	Plaine Magnien	55	678409	p	1.08	816, 747
21	Plaine Magnien (EDC)	76	678412	s	0.52	825
22	Carreau Esnouf	80	682391	wcb	—	745
23	Carreau Cassia	80	686374	wcb	0.65	743
24	Union Vale	100	688388	wcb	0.48	824
Savanne						
25	Bel Air St. Félix	58	596328	wcb	0.66	746, 826, 877

[1]All grid references are taken from series Y682, sheet Mauritius and edition 1.4545. References are for guidance only, exact locality details can be obtained from the Ministry of Health, Mauritius.
[2]cdc: concrete drainage channel; sp: small pool; p: pool; m: marsh; wcb: water cress beds.

upper whorls. This feature was common in populations from the Grand Port district (19–24) and was seen in samples 9, 15, 16, 17 and 18. No decollate snails were observed in the F_1 generation of samples reared in the laboratory. Variation in the size and shape of the shell was apparent; representatives of 12 of the populations sampled are shown in Fig. 3. Many specimens were ribbed but this does not seem to be a consistent feature. Analysis of measurements of 50 snails from each of samples 7, 12, 14 and 23 (Cité la Cure, Trou aux Biches, Cap Malheureux and Carreau Cassia) showed marked differences in the ratios of shell height to height of the aperture. The larger, shell height to aperture height ratios of sample 12 clearly differentiated it from the others at shell lengths over 4 mm; shells from sample 23 had the smallest ratios at all shell lengths (only complete shells were used); samples 7 and 14 lay between the two extremes.

The snails responded differently to laboratory conditions, but observation of subsequent generations suggested that a characteristic shell form was maintained. Measure-

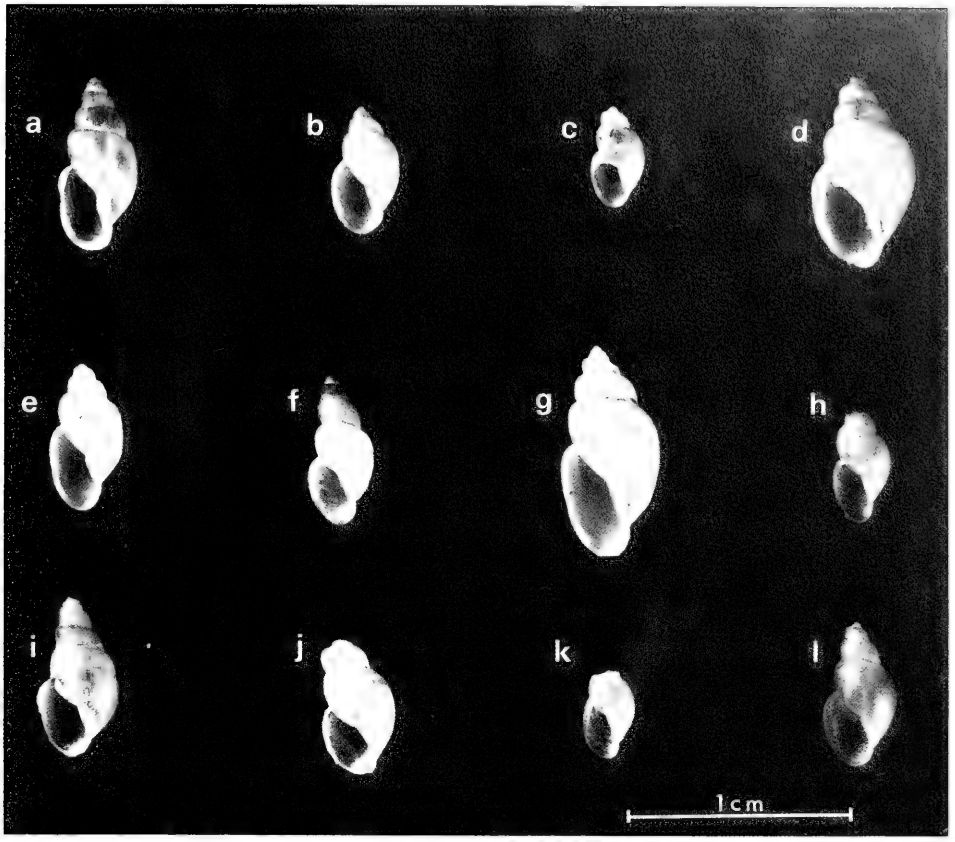

FIG. 3. Shells of *B. cernicus*; a, Chateau d'Eau; b, La Paix Stream; c, Pailles; d, Balaclava; e, Trou aux Biches; f, Cap Malheureux; g, Roches Noires; h, Clémencia; i, Mare d'Albert; j, Plaine Magnien; k, Carreau Cassia; l, Bel Air St. Félix.

ments, taken at weekly intervals from hatching to 15 weeks, of 10 snails from sample 4 (Pouce Stream) and 10 from sample 12 (Trou aux Biches) showed that snails from sample 12 maintained a greater height of shell to height of aperture ratio at all shell sizes. The greatest percentage increase in shell height occurred in the second week of growth, 50% for sample 4 and 49% for sample 12. Egg-laying by sample 4 commenced at 8½ weeks at an average shell height of 4.25 mm but did not begin until 11½ weeks in sample 12 at an average shell height of 5.75 mm.

Radula

Radulae from snails representing 14 samples (3, 4, 5, 6, 7, 9, 11, 12, 13, 16, 17, 20, 22, 23) have been examined by SEM; for comparative purposes, an attempt was made to standardise the size of the snail from which the radula was dissected. Undoubtedly, differences exist between the populations but due to the finding of many intermediates between the two extremes, designated as the "northern" and "southern" types, it was difficult to categorise the different kinds (Fig. 4). The "southern" type, particularly associated with snails from Grand Port and Flacq districts, had more elongate cusps and greater separation between the mesocones and endocones.

Snail susceptibility

Only three of the snails proved to be carrying natural infections of *S. haematobium*; these snails were all collected from the Vallée Pitot site. Thirteen of the populations have been tested for susceptibility to a freshly isolated Mauritian strain of *S. haematobium* and

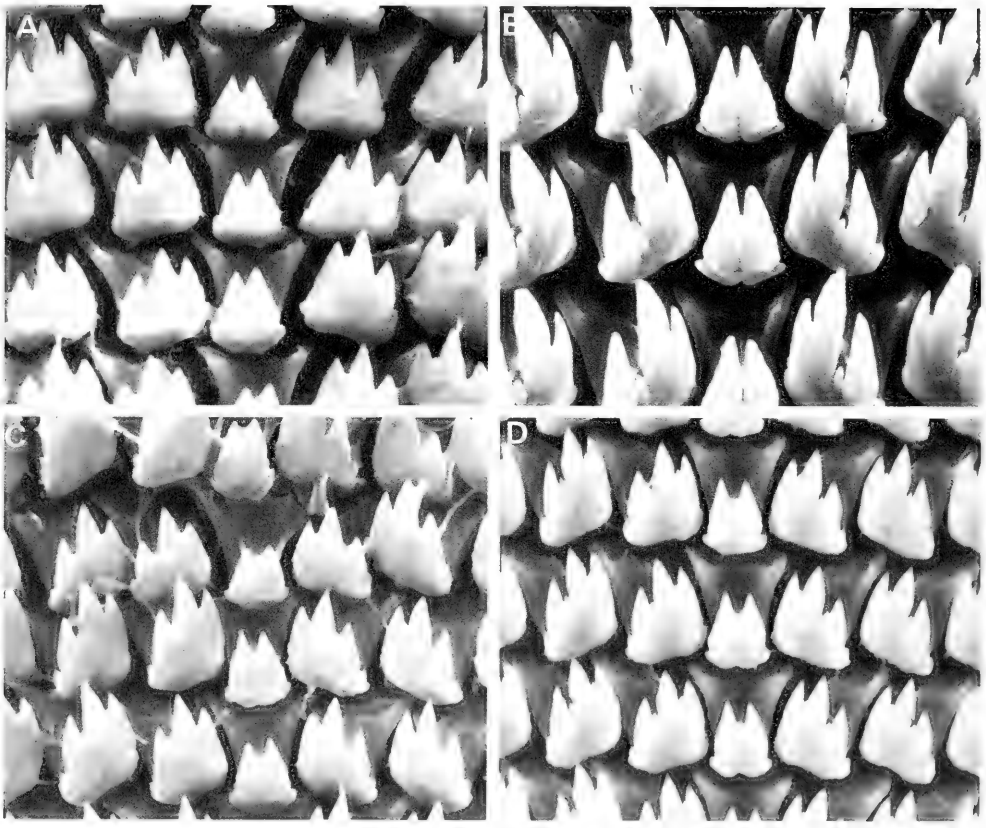

FIG. 4. Stereoscan pictures of *B. cernicus* radulae; A, Dauguet (northern type); B, Carreau Cassia (southern type); C, Pailles (intermediate); D, Grande Baie (intermediate).

TABLE 2. Experimental infection of *B. cernicus* with *S. haematobium* (Mauritius).

Sample no.	Locality	No. of snails exposed	No. of survivors	% survivors infected[1]	% exposed infected[1]
2	Chateau d'Eau	25	24	91.7	88
3	Dauguet	25	19	68.4	52
4	Pouce Stream	50	5	60	6
5	Vallée Pitot	25	21	85.7	72
6	La Paix Stream	30	26	69.2	60
7	Cité la Cure	50	49	28.8	28
11	Balaclava	25	12	58.3	28
12	Trou aux Biches	50	44	68.2	60
13	Grande Baie	25	23	69.6	64
14	Cap Malheureux	85	68	47.1	37.6
16	FUEL	30	25	60	50
18	Clémencia	20	19	42.1	40
24	Union Vale	35	20	65	37.1

[1]Based on enzyme data and cercarial shed.

TABLE 3. Experimental infection of *B. cernicus* with *Schistosoma* spp.

Sample no.	Locality	No. of snails exposed	No. of survivors	% survivors infected[1]	% exposed infected[1]
S. intercalatum (Cameroun)					
5	Vallée Pitot	25	15	100	60
12	Trou aux Biches	40	37	43.2	40
14	Cap Malheureux	40	39	92.3	90
17	Bel Air R. Sèche	24	17	94.1	66.7
18	Clémencia	40	16	75	30
20	Plaine Magnien	8	6	100	75
S. intercalatum (Gabon)					
13	Grande Baie	50	21	85.7	36
S. bovis (Kenya)					
15	Roches Noires	50	43	53.5	46
S. haematobium (Sudan)					
2	Chateau d'Eau	25	21	4.8	4
5	Vallée Pitot	25	10	30	12
S. haematobium (Kenya)					
5	Vallée Pitot	25	17	52.9	36

[1]Based on enzyme data and cercarial shed.

the results are shown in Table 2. This laboratory isolate was established using eggs from 6 urines collected from children in the Grand Port district. Populations of *B. cernicus* have also proved to be susceptible to *S. intercalatum* (Cameroun and Gabon), *S. bovis* (Kenya) and *S. haematobium* (Sudan and Kenya); the results are given in Table 3. Schistosome infections in the snails were detected by cercarial shed and by electrophoretic analyses of homogenates of snail digestive gland containing the developing parasites (Fig. 5). No correlation was observed between the alleles identified in the samples and susceptibility.

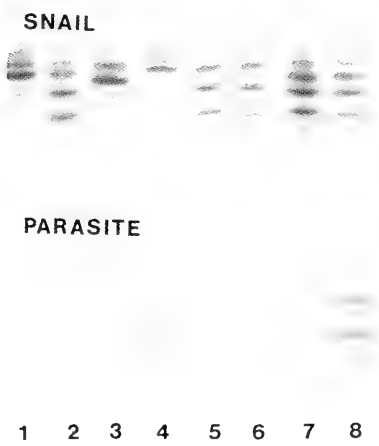

FIG. 5. GPI separation on pH 4-9 gel of extracts from snails (Clémencia, sample 18) previously exposed to *S. intercalatum* (Cameroun). Snails 2, 3, 4, 5, 7, and 8 were infected.

Enzyme analyses

Snails from all samples were analysed at 6 enzyme loci: MDH, PGM, GPI, AcP-A, AcP-B and HBDH. The designation of the enzyme types was based on the pI values of the main bands of enzyme activity, the numbering of types beginning with the most alkaline. No relationship is implied with systems previously described for other species of *Bulinus*. Where comparisons have been made, it seems that isoelectric focusing will often reveal more bands of enzyme activity than starch gel and although many of the enzyme types consist of a number of enzyme bands, it is possible to equate them with alleles or allozymes. The occurrence of the enzyme types is detailed in Table 4 and the types illustrated in Fig. 6. Where heterozygotes have been identified in the wild-caught snails, they have either been confirmed by subsequent genetic crosses or, if not, have been assumed.

TABLE 4. Enzyme types encountered in populations of *B. cernicus*.

Sample no.	Locality	MDH	PGM	GPI	AcP-A	AcP-B	HBDH
1	Canal d'Etang	2	1	2,4	2	4	4
2	Chateau d'Eau	2	1	4	2	4	4
3	Dauguet	2	1	2,4	1,2	3,4	1,4
4	Pouce Stream	2	1	2,4,6	2	4	4
5	Vallée Pitot	2	1	2,4,6	2	3,4	4,5
6	La Paix Stream	2	1	2,3,4,6	1,2	3,4	1,4,5
7	Cité la Cure	2	1	2,4	2	4	1,4
8	Canal Caudan	2	1	2,6	2	4	4
9	Pailles	2	1	2,6	2	4	2,4
10	Arsenal	2	1	2,4	2	4	4
11	Balaclava	2	1	2,3,4	2	4	4
12	Trou aux Biches	1,2	1	4	2	3,4	4
13	Grande Baie	2	1	4	2	3,4	4
14	Cap Malheureux	2	1	4	2	3,4	4
15	Roches Noires	2	1	2,4	2,3	3,4	4
16	FUEL	2	1	2,4,5,6	2	2,4	4
17	Bel Air R. Sèche	2	1	2,3,5,6	2	1,3,4	4
18	Clémencia	2	1	1,2,3,4,6	2	2,4	3,4
19	Mare d'Albert	2	1	6	2	4	4
20	Plaine Magnien	2	1	6	2	4	4
21	Plaine Magnien EDC	2	1	6	2	4	4
22	Carreau Esnouf	2	1	6	2	4	4
23	Carreau Cassia	2	1	6	2	4	4
24	Union Vale	2	1	6	2	4	4
25	Bel Air St. Félix	2	1	2,4,5,6	2,4	1,2,4	3,4

MDH

Two enzyme types have been identified, MDH-1 and MDH-2; four bands of enzyme activity made up the patterns which occurred between pH 8.4 and 6.0. With the exception of sample 12, the only population in which MDH-1 was seen, all the populations were monomorphic for MDH-2. The frequency of the MDH alleles based on forty snails from sample 12 was MDH-2, 0.875 and MDH-1, 0.125. The pattern of the heterozygote suggested a monomeric enzyme.

PGM

PGM-1 consisted of four major bands of activity between pH 6.0 and 5.4 and was found to be invariant among the snails examined.

GPI

Six enzyme types have been recognised for GPI; the pattern consists of two major bands. Due to the very close banding of the GPI types, all resolving within the pH range 5.8 to 5.3, they could only be satisfactorily distinguished using a 4–6 pH gel and an appropriate marker (Fig. 7). The enzyme appears to be a dimer. The frequencies of the alleles are given in Table 5 and the pattern of distribution around the island is indicated in Fig. 8.

AcP-A

Two AcP loci have been recognised as shown in Fig. 9, the four segregating alleles of AcP-A falling between pH 6.9 and 6.4. All populations surveyed had AcP-2, while the other alleles were confined to particular populations; the frequencies are given in Table 6.

AcP-B

Four enzyme types were identified, consisting of one major band of activity falling between pH 5.6 and 4.9. AcP-4 was common throughout and the only allele identified in

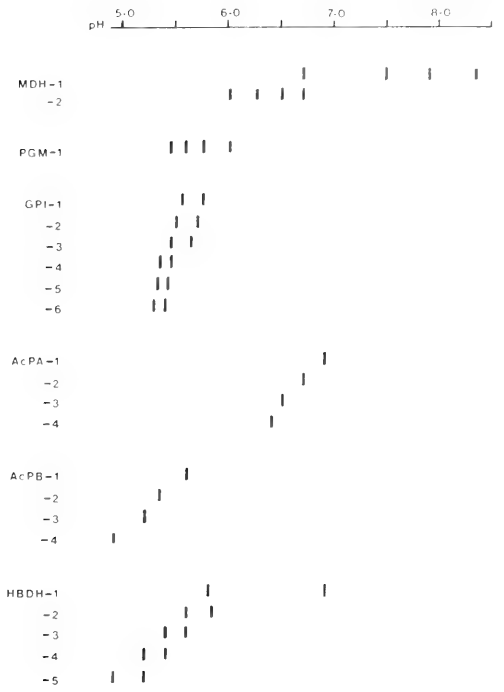

FIG. 6. Diagrammatic representation of enzyme types identified in *B. cernicus.*

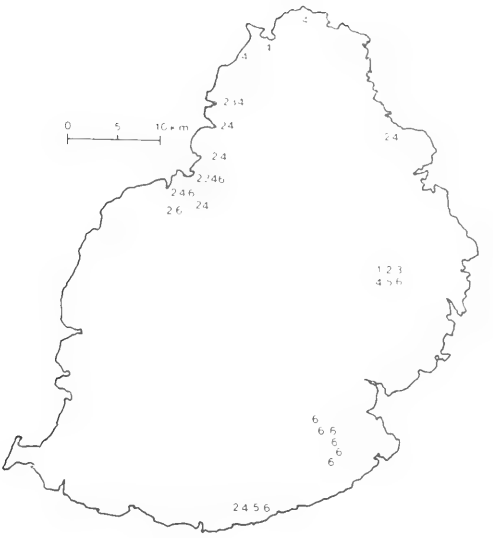

FIG. 8. Map of Mauritius showing distribution of GPI alleles.

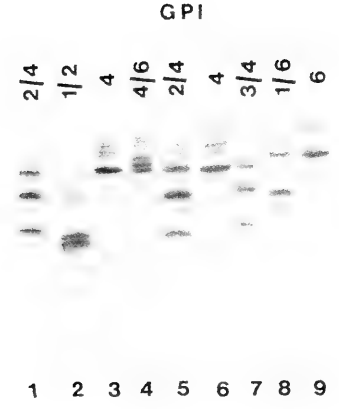

FIG. 7. GPI separation on pH 4–6 gel of 9 snails from Clémencia, sample 18.

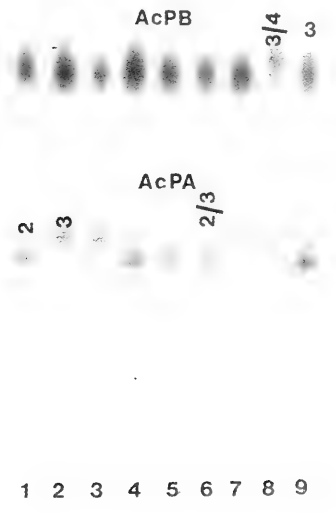

FIG. 9. AcP separation on pH 4–9 gel of nine snails from Roches Noires, sample 15.

TABLE 5. Allelic frequencies at the GPI locus.

Sample no.	Locality	N	GPI-1	GPI-2	GPI-3	GPI-4	GPI-5	GPI-6
1	Canal d'Etang	2		0.750		0.250		
2	Chateau d'Eau	25				1.000		
3	Dauguet	16		0.375		0.625		
4	Pouce Stream	18		0.750		0.111		0.139
5	Vallée Pitot	40		0.325		0.525		0.150
6	La Paix Stream	45		0.533	0.044	0.133		0.289
7	Cité la Cure	31		0.194		0.806		
8	Canal Caudan	8		0.625				0.375
9	Pailles	14		0.071				0.929
10	Arsenal	5		0.700		0.300		
11	Balaclava	20		0.325	0.425	0.250		
12	Trou aux Biches	26				1.000		
13	Grande Baie	10				1.000		
14	Cap Malheureux	44				1.000		
15	Roches Noires	30		0.017		0.983		
16	FUEL	28		0.732		0.125	0.107	0.036
17	Bel Air R. Sèche	11		0.636	0.045		0.045	0.272
18	Clémencia	35	0.100	0.186	0.086	0.329		0.300
19	Mare d'Albert	10						1.000
20	Plaine Magnien	20						1.000
21	Plaine Magnien EDC	10						1.000
22	Carreau Esnouf	10						1.000
23	Carreau Cassia	4						1.000
24	Union Vale	10						1.000
25	Bel Air St. Félix	50		0.265		0.422	0.206	0.108

TABLE 6. Allelic frequencies at the AcP-A locus.

Sample no.	Locality	N	AcP-A-1	AcP-A-2	AcP-A-3	AcP-A-4
3	Dauguet	40	0.325	0.675		
6	La Paix Stream	35	0.100	0.900		
15	Roches Noires	20		0.450	0.550	
25	Bel Air St. Félix	31		0.726		0.274

TABLE 7. Allelic frequencies at the AcP-B locus.

Sample no.	Locality	N	AcP-B-1	AcP-B-2	AcP-B-3	AcP-B-4
3	Dauguet	40			0.425	0.575
5	Vallée Pitot	10			0.650	0.350
6	La Paix Stream	44			0.364	0.636
12	Trou aux Biches	75			0.387	0.613
13	Grande Baie	10			0.400	0.600
14	Cap Malheureux	43			0.384	0.616
15	Roches Noires	30			0.817	0.183
16	FUEL	12		0.125		0.875
17	Bel Air R. Sèche	11	0.136		0.091	0.773
18	Clémencia	36		0.167		0.833
25	Bel Air St. Félix	34	0.059	0.117		0.824

fourteen populations. AcP-3 occurred in sample 17 and certain populations in the North, whereas AcP-2 and AcP-1 were restricted to samples 16, 18, 25 and 17, 25 respectively. The enzyme behaved as a dimer; the allelic frequencies are given in Table 7.

HBDH

Five HBDH enzyme types, consisting of two bands of enzyme activity, occurred between pH 6.9 and 4.9. Further HBDH activity, often present at the alkaline side of the gel, probably represented another locus; however, the expression was too inconsistent to be of use. HBDH-4 was common throughout; eighteen populations were monomorphic for this enzyme. HBDH-1 and HBDH-5 were associated with populations in and around Port Louis, HBDH-2 was unique to sample 9 and HBDH-3 was found in samples 18 and 25. The enzyme appears to be a dimer; the allelic frequencies are given in Table 8.

The observed distributions at each locus in examples where over 15 individuals were analysed and two alleles identified, were examined for their goodness of fit to Hardy-Weinberg expectations. No significant deviations were found. The mean heterozygosity per locus ranged from H = 0 to 0.265 with an overall mean of H = 0.09.

Isolation of lines

GPI

Ten wild-caught snails from sample 6 were isolated, 5 of which produced eggs (Lines A,B,C,D, and E). The young snails were isolated as hatchlings and produced an F_2 generation. The details are shown in Fig. 10. Of the original isolated parents, at least four carried donated sperm, as new alleles were found in the F_1 generation (GPI-3 in line A, GPI-6 in line B, GPI-4 in line D and GPI-6 in line E). With the F_1 generation, self-fertilising homozygotes produced homozygotes whereas self-fertilising heterozygotes produced both homozygotes and heterozygotes. F_2 snails homozygous for GPI-2 in line A were used in subsequent crosses.

TABLE 8. Allelic frequencies at the HBDH locus.

Sample no.	Locality	N	HBDH-1	HBDH-2	HBDH-3	HBDH-4	HBDH-5
3	Dauguet	40	0.175			0.825	
5	Vallée Pitot	40				0.900	0.100
6	La Paix Stream	37	0.243			0.743	0.014
7	Cité la Cure	24	0.104			0.896	
9	Pailles	15		0.067		0.933	
18	Clémencia	36			0.083	0.917	
25	Bel Air St. Félix	10			0.100	0.900	

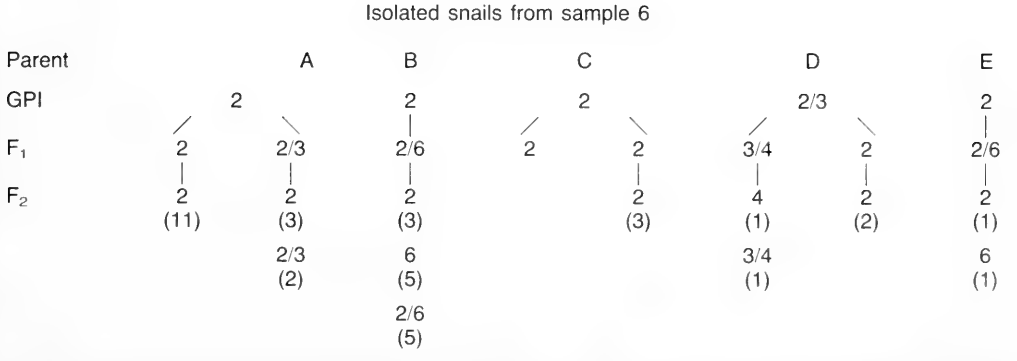

FIG. 10. Isolated snails from La Paix Stream and their progeny. Numbers in parentheses represent numbers of snails produced and analysed.

Similar isolations of sample 4 yielded offspring in the first generation which indicated that prior cross-fertilisation had taken place. One parent homozygous for GPI-2 produced five 2/6 heterozygotes and one 2/4 heterozygote, suggesting a possible mating with a 4/6 heterozygote. Such a snail was not seen in the laboratory colony from which these snails were isolated and hence this may be an indication of fertilisation taking place before collection, eighteen days prior to isolation or possibly of multiple insemination.

Crosses

1. Trou aux Biches (12) × Carreau Esnouf (22)

Ten pairings were set up utilising juvenile snails. All the snails from sample 22 died in the first two weeks. One of the snails from sample 12 proved to be heterozygous for MDH; MDH-2 and MDH-1/2 were identified in the F_1.

2. Cap Malheureux (14) × Plaine Magnien (20)

Two of the ten pairings set up produced eggs. The parentals from sample 14 were characterised by GPI-4 and those from sample 20 by GPI-6 and eight F_1 examined proved to be GPI 4/6 heterozygotes. Eight F_1 from one cross were kept together and laid numerous egg masses; not all the eggs developed and only a small number of F_2 was produced. Ten of the F_2 were analysed: two GPI-4, two GPI-6 and six GPI-4/6 snails were identified. The F_1 snails from the other pairing did not produce eggs during five months of observation. Radulae from the F_1 snails were examined; they appeared to be intermediate as they could not readily be identified as either 'northern' or 'southern-type.'

3. Cap Malheureux (14) × Union Vale (24)

Four pairings were set up utilising juvenile snails; in all cases, the Union Vale partner died before commencement of egg-laying and all young snails subsequently produced by the parentals from sample 14 were of the same enzyme type, GPI-4.

Sperm storage

La Paix (6) × Trou aux Biches (12)

Ten pairings were set up using snails less than 3 mm; the parentals from sample 6 possessed GPI-2 whereas GPI-4 characterised sample 12. Eggs were produced by all the pairs; of the ten, the F_1 from eight were found to be heterozygotes with GPI-2/4; the other two produced GPI-2 F_1. Five of the crosses which produced heterozygotes (1–5) were retained. Each parent was isolated and moved into a new container to continue egg-laying. All the F_1 produced by each parental snail were analysed for GPI and the adult snails

	1		2		3		4		5	
Parents	6 × 12		6 × 12		6 × 12		6 × 12		6 × 12	
F_1	2/4 (11)		2/4 (8)		2/4 (10)		2/4 (6)		2/4 (15)	
Parent	6	12	6	12	6	12	6	12	6	12
Day	|	|	|		|	|	|		|	
0–21	2/4 (10)	2/4 (9)	2/4 (6)	died	2 (6)	2/4 (4)	2/4 (12)	—	2/4 (10)	—
22-37	2/4 (10)	—	—		2 (5)	2/4 (3)	2/4 (12)	—	2/4 (8)	—
38-49	2/4 (1)	—	—		—	—	2/4 (3)	—	—	—
50–70	2/4 (1)	—	—		—	—	2/4 (2)	—	2/4 (3)	—
71–80	—	—	—		—	—	2 (2)	—	2 (3)	—

FIG. 11. GPI-types in the F_1 of the 6 × 12 cross after separation of the parentals. Numbers in parentheses represent numbers of snails produced and analysed.

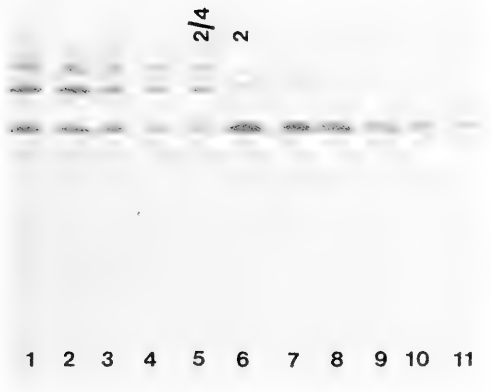

FIG. 12. GPI separation on pH4–6 gels of the F_1 from the 6 × 12 cross showing results of cross- (GPI-2/4) and self-fertilisation (GPI-2).

were moved to new containers after egg-laying. The results are depicted in Fig. 11, the enzyme differences in Fig. 12. Heterozygotes, GPI-2/4, were produced for up to 70 days after isolation by three of the parents; two of the snails then reverted presumably to self-fertilisation and produced homozygotes, GPI-2 in both cases. The snails from sample 6 were cross-fertilised in four of the crosses whereas only two snails from sample 12 showed evidence of cross-fertilisation by producing heterozygotes; the donated sperm in these cases was not utilised after 37 days. Two of the snails from sample 12 survived for fourteen weeks without producing offspring of any kind. In only one pairing did cross-fertilisation occur both ways.

DISCUSSION

The volcanic activity that built up the Mascerene plateau gave rise to Mauritius about seven million years ago in the early Pliocene. The presence of raised reefs overlying basaltic strata suggested to Simpson (1950) that volcanic activity must have ceased in Mauritius more than 100,000 years ago, a finding corroborated by MacDougall and Chamalaun (1969). Mauritius is the oldest of the three islands comprising the Mascerenes and the only one at present to be successfully colonised by a species of *Bulinus*.[1] The freshwater molluscs in the Mascerene island group have been studied most recently by Starmühlner (1976 and 1977): some of the species appear to be related to Asian molluscs as mentioned by Brown (1980), whereas the relationships of *B. cernicus* and others most clearly lie with the African fauna. Although *B. cernicus* belongs to the *forskali* group of bulinids, little is known concerning affinities within the group. A more detailed examination of *B. forskali* and *B. bavayi* from Madagascar is needed but preliminary enzymatic and morphological evidence would favour a closer relationship between *B. cernicus, B. scalaris* (Dunker, 1845) and *B. bavayi* than between *B. cernicus* and *B. forskali* (Wright & Rollinson, unpublished). When and how *Bulinus* reached Mauritius remains unknown in the absence of fossil evidence. As surface currents in the south-west Indian Ocean do not appear to favour movement of drift from the African mainland to Mauritius (Martin, 1981), migratory birds acting as carriers may have been responsible for the introduction of *Bulinus*. The arrival of man and *S. haematobium* has been comparatively recent, permanent settlements not beginning until the early eighteenth century.

The island is approximately 60 km along the major axis (NNE-SSW) and 45 km along the minor axis. The Ministry of Health, Mauritius, has to date recorded between 55 and 60 habitats for *B. cernicus*. It is probable that the attention given to the snail, since the appreciation of its rôle as an intermediate host, may well account for the increase in the number of sites from which *B. cernicus* has been recorded in recent years. However, man's influence on the environment must have altered the snail's distribution; concrete drainage channels, water cress beds and pools in sugarcane fields are much favoured habitats.

B. cernicus is well adapted to the subtropical climatic conditions on Mauritius; from December to April, rainfall is highest and cyclones may occur; between October and January, there may be periods of drought. In regions exposed to floods, many snail populations will be scoured out from their breeding places and, in times of drought, only those snails which successfully aestivate will survive. *B. cernicus* appears to be remarkably resistant to drought. Reports of survival in dried-up habitats of 28 months (Mamet, 1968)

[1]An isolated occurrence of *B. cernicus* has recently been discovered on Reunion (Julvez, Ministry of Health, Reunion, personal communication) but this has yet to be confirmed by the examination of live material.

and 36 months (Courtois & Gébert, 1979) have been recorded. Population size must fluctuate dramatically under these conditions. Figures on snail densities for two sites, Carreau Cassia and Cité la Cure, show that the highest population densities are to be found in the middle of the year (Courtois & Gébert, 1979). Even at these peak times, snails may not be found in some habitats. The survey in 1980 during May and June failed to discover *B. cernicus* in 28 out of 50 known habitats examined.

Genetic drift might well be expected to operate in isolated populations that are drastically reduced during certain seasons of the year and, indeed, one of the most striking features about *B. cernicus* in comparison to other bulinids is the morphological and enzymatic differences found between populations. The preliminary results on shell measurements indicate marked differences in the shape and size of the shell between populations and despite an obvious environmental component to this variation, the characteristic shell form appears to be maintained in snails reared in the laboratory. In general, smaller squatter snails, often decollate, are common in habitats from the Grand Port district, Bel Air St Félix, Clémencia, Bel Air Riviere Sèche and Pailles, in contrast to the more slender, higher spired forms of Trou aux Biches and Cap Malheureux. A more detailed morphometric analysis would seem worthwhile to appreciate the differences between populations. Variation in the radular teeth appears continuous and is difficult to delimit. The greatest differences again appear to occur between populations from the N.W. and S.E. of the island. The teeth of the snail from the S.E. are characterised for the most part by having more elongated cusps with greater separation between the mesocone and endocone. Little is known concerning the inheritance of these characters. In one of the crosses using snails from the two areas (Cap Malheureux (14) × Plaine Magnien (20)), the radular teeth of the F_1 were examined. The teeth were classed as intermediate, as they did not resemble the teeth of either parent.

The appearance of decollate shells in some populations appears to be correlated with low calcium levels; no decollate shells were produced in F_1 generations reared in water with high levels of calcium. It is probable that environmental differences other than calcium occur between the habitats. The samples from Grand Port, Flacq, Moka and Savanne districts proved difficult to maintain in the laboratory, as judged by survival and egg production, even when water of low calcium concentration was used. These snails are often associated with water cress beds which are characterised by slow-flowing water and volcanic vesicular rock on which the snails are found.

Although only six enzyme loci have been examined, the amount of enzyme variation was surprising in the light of studies on other members of the *B. forskali* group, which have proved fairly conservative (Wright *et al.*, 1979; Jelnes, 1980). No obvious diagnostic alleles were found in support of groupings based on shell morphology or radular teeth differences, although marked differences in the frequency and distribution of alleles occurred. For example, although all populations in the Grand Port district were monomorphic for GPI-6, this allele also occurs in nine other populations in the Port Louis, Moka, Flacq and Savanne districts. Similarly, GPI-4 characterised three of the populations in the north of the island (12, 13, 14) but is common elsewhere. The mean heterozygosity per locus ranged from $H = 0$ to 0.265 with seven populations proving invariant for all the enzyme loci. With the exception of sample 2, Chateau d'Eau, all the invariant samples were collected in the Grand Port district. Sample 2 was based on 23 snails collected in small pools in the process of drying out, whereas the samples from Grand Port were based on larger numbers from water cress beds, a stream and two pools.

One of the interesting findings was that of the 22 alleles identified, five (MDH-1, GPI-1, AcP-A-3, AcP-A-4 and HBDH-2) were restricted in their distribution to a particular population (12, 18, 15, 25, 9, respectively) and four (AcP-A-1, AcP-B-1, HBDH-3 and HBDH-5) were only present in two populations (3 & 6, 17 & 25, 18 & 25, 5 & 6, respectively). Most of these alleles occurred at a low frequency in the populations in which they were found. The samples possessing unique alleles represented some of the more isolated habitats. Although random genetic drift may be responsible for many of the observed differences, nothing is known of the selection pressures that might be operating on these enzyme polymorphisms. It seems that gene flow between habitats is restricted to a very low level. Where an allele was found in only two populations, the habitats were either geographically close, as samples 3 and 6 and

samples 5 and 6 in Port Louis, or linked by some other factor. Sample 25, Bel Air St. Félix, in the south, shares a number of alleles with the three samples (16, 17, 18) from Flacq in the east (AcPB-1, HBDH-3, GPI-5); it is probable that snails have been transported with water cress between the two districts. Mamet (1968) remarked upon the finding of snails on cress at markets in the towns and the possibility of transferring snails from one part of the island to another.

There is little comparative information concerning the breeding biology of *Bulinus*, although different species probably employ different reproductive strategies. Given the limitations of the collections and the enzyme analyses, it seems that at the time of sampling, the snails were the result of cross-fertilisation as the observed distributions at each locus tested did not deviate from Hardy-Weinberg expectations. Further evidence of cross-fertilisation was provided by the isolation of wild-caught snails; four snails out of five isolated from sample 6 produced eggs which were indicative of the parent carrying donated sperm. No evidence of multiple insemination was seen. In the laboratory mating experiments between snails from Trou aux Biches (12) and La Paix (6), the pairings showed a marked preference for cross-fertilisation; eight of the crosses produced heterozygotes, whereas the eggs produced in the other two were the result of self-fertilisation.

As the greatest differences seemed to lie between populations from the N.W. and S.E., attempts were made to test if there was any evidence of reproductive isolation between individuals representing the two areas. Only two out of twenty-four pairings produced F_1 by cross-fertilisation; this was in marked contrast to the Trou aux Biches (12), La Paix (6) cross where eight out of ten were successful. The difficulty in maintaining the snails from the S.E. probably accounts for part of this anomaly; the snails from the S.E. died in 14 of the 24 pairings, although a comparable death rate was not noticed in the stock trays. In unsuccessful crosses, it is not known whether copulation takes place or whether some post-mating barrier is responsible. The F_1 snails produced by the N.W./S.E. cross were fertile in one instance. It seems that although reproductive isolation is not complete, marked differences exist in the reproductive success of pairings between snails from different populations.

The results from isolating parents after mating showed that sperm can be stored for up to 70 days and that donated sperm was depleted before reversion to self-fertilisation, at least in the Trou aux Biches (12), La Paix (6) cross. There appears to be no mixing of cross- and self-fertilisation, although it is by no means clear how the switch from one method to the other takes place. In *Bulinus*, as in other planorbids, the ova and sperm are produced together in the ovotestis, pass out into the lumen of the ovotestis and travel down the hermaphrodite duct together. Some mechanism must operate to stop self-fertilisation in the presence of donated sperm to allow cross-fertilisation, which appears to be preferred, to take place. Evidence for sperm storage was noted by Wu (1972) for *Bulinus tropicus* using albinism as a marker. The period of cross-fertilisation varied from two to nine weeks after isolation and the transitional period varied from two to three weeks. Wu (1972) sugested that the switching from cross- to self-fertilisation depended on the quantity of donated sperm received during copulation and that the quantity depended on the frequency and duration of copulation. Similar observations on sperm storage have been recorded by Paraense (1955) for *Biomphalaria glabrata* and by Mulvey and Vrijenhoek (1981) for *Biomphalaria obstructa*.

Sperm storage allows cross-fertilisation over long periods without repeated copulation and may be a mechanism for maintaining heterogeneity during aestivation or colonisation; as each snail may not only be carrying its own genes but also those of another individual. In the five 12 × 6 crosses that were monitored, cross-fertilisation only occurred both ways in one cross. Previous suggestions have been made that species of *Bulinus* may be protandrous (Wright, 1957; Wu, 1972) and it may be that not all the snails used in the cross had reached the same reproductive state by the time of separation. If protandry does occur in *B. cernicus,* it may be possible for a snail to receive sperm and store it awaiting the production of ova; furthermore, there might be an association between self-fertilisation and the age of the snail.

Frandsen (1979) reported differences in susceptibility to *S. intercalatum* from Cameroun between samples of *B. cernicus* originating from Cité la Cure and Clémencia (percent survivors infected were 17.1% and 79.3%, respectively). When this parasite was used in

the present study, 75% of the survivors from Clémencia were infected and a range in susceptibility was seen in the other samples tested (43% to 100% of the survivors were infected). Snails from Cité la Cure were not tested for susceptibility to *S. intercalatum* but showed a correspondingly low infection rate (28.6% of the survivors infected) when exposed to a local strain of *S. haematobium*. An important finding was that all of the thirteen samples tested were susceptible to the local strain, although differences in the percentage of survivors infected ranged from 28.6% to 91.7%. Susceptibility to schistosomes is known to be under genetic control and it is possible that the genes responsible occur at different frequencies within the population. No correlation was apparent between known transmission sites and high or low susceptibility rates in the corresponding snail samples. For example, of two known transmission sites, Cité la Cure had the lowest experimental infection rates for *S. haematobium* whereas Vallée Pitot had the second highest. It is possible that differences also exist in parasite infectivity between populations of *S. haematobium* on Mauritius; the parasite used for these experiments was isolated from patients living in the S.E. of the island. There were, however, no marked differences in the infection rates of snails from this area compared to others. Michelson and DuBois (1981) have shown a strong correlation between the frequency of certain acid phosphatase isoenzymes observed in populations of *Biomphalaria glabrata* and the level of susceptibility to *S. mansoni*. In the present study, no correlation was observed between any of the alleles identified and susceptibility to schistosomes. In any future control programme for schistosomiasis on the island, it would be important to take the breeding biology of *B. cernicus* into account. The ability to withstand large reductions in population numbers suggests that the snail would prove to be a formidable opponent for any mollusciciding programme.

In conclusion, *B. cernicus* is a species well adapted to a variety of temporary and semi-permanent habitats. The habitats are for the most part isolated and the populations have diverged in morphological, enzymatic and other biological characters; discrete gene pools probably suggest restricted gene flow between habitats. It is, as yet, a perplexing problem as to why this snail inhabiting such a small area should show more variation than other members of the *B. forskali* group, some of which occur over a very much larger geographical area. It is hoped that more detailed studies on other species of this group will give further insight into this intriguing situation. The snails are capable of self-fertilisation, which may well be employed at certain times, but the evidence suggests that cross-fertilisation is preferred and the usual manner of reproduction. Sperm storage may be an important method of maintaining heterogeneity at times of low population density and ensuring cross-fertilisation without repeated copulation. The mating experiments revealed marked differences in the success of different pairings, although fertile F_1 snails were produced. The significance of these 'no-choice' pairings might be questioned but, if nothing else, they again reveal inherent differences between populations. It is unnecessary to name new taxa for the forms that can now be found on Mauritius, but to consider them as belonging to one variable species. This is satisfactory from the practical viewpoint as all samples tested were susceptible to *S. haematobium*. The present study has allowed a glimpse at the variability and breeding biology of one species of *Bulinus*; further work is needed in order to help answer some of the questions pertinent to understanding the evolution of this important group of snails.

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REFERENCES CITED

BETTERTON, C., FRYER, S. E. & WRIGHT, C. A., 1983, *Bulinus senegalensis* (Mollusca: Planorbi-

dae) in northern Nigeria. *Annals of Tropical Medicine and Hygiene,* 77: 143–149.

BIOCCA, E., BULLINI, L., CHABAUD, A. G., NASCETTI, G., ORECCHAI, P. & PAGGI, L., 1979, Subdivisione su base morfologica e genetica del genere *Bulinus* in tre generi: *Bulinus* Muller, *Physopsis* Krauss e *Mandahlbarthia* gen. nov. *Rendiconti della Classe di Scienze fisiche, matemátiche et naturali, Accademia Nazionale dei Lincei,* ser. 8, 6: 276–282.

BROWN, D. S., 1980, *Freshwater snails of Africa and their medical importance.* Taylor & Francis, London, x + 487 p.

COURTOIS, C. M. & GÉBERT, F., 1979, Recent observations on schistosomiasis in Mauritius. *Tropical and Geographical Medicine,* 31: 381–387.

COWPER, S. G., 1953, Schistosomiasis in Mauritius. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 47: 564–579.

FRANDSEN, F., 1979, Further studies on the compatibility between *S. intercalatum* from Cameroun and Zaïre and species of *Bulinus. Zeitschrift für Parasitenkunde,* 58: 161–167.

GITELMAN, H. J., 1967, An improved automated procedure for the determination of calcium in biochemical specimens. *Analytical Biochemistry,* 18: 521–531.

JELNES, J. E., 1977, An electrophoretic character useful in the distinction between *Bulinus tropicus* and *B. permembranaceus* (Gastropoda: Planorbidae). *Steenstrupia,* 4: 139–141.

JELNES, J. E., 1978, Experimental taxonomy of *Bulinus* (Gastropoda: Planorbidae). I. Electrophoretic studies on esterase and phosphoglucose isomerase of *Bulinus truncatus.* Origin, distribution, geographical variation and taxonomic implications. *Archiv für Molluskenkunde,* 109: 237–248.

JELNES, J. E., 1979, Experimental taxonomy of *Bulinus* (Gastropoda: Planorbidae). II. Recipes for horizontal starch gel electrophoresis of ten enzymes in *Bulinus* and description of internal standard systems and of two new species of the *Bulinus forskalii* complex. *Journal of Chromatography,* 170: 403–411.

JELNES, J. E., 1980, Experimental taxonomy of *Bulinus* (Gastropoda: Planorbidae). III. Electrophoretic observations on *Bulinus forskalii, B. browni, B. barthi* and *B. scalaris* from East Africa, with additional electrophoretic data on the subgenus *Bulinus* sensu stricto from other parts of Africa. *Streenstrupia,* 6: 177–193.

MacDOUGALL, I. & CHAMALAUN, F. H., 1969, Isotopic dating and geomagnetic polarity studies on volcanic rocks from Mauritius, Indian Ocean. *Geological Society of America Bulletin,* 80: 1419–1442.

MAMET, J. R., 1968, La bilharziose vésicale l'Ile Maurice et son hôte intermediaire, *Bulinus cernicus* Morelet. *Proceedings of the Royal Society of Arts and Sciences of Mauritius.* 3: 75–100.

MANDAHL-BARTH, G., 1957, Intermediate hosts of *Schistosoma,* African *Biomphalaria* and *Bulinus.* 2. *Bulinus. Bulletin of the World Health Organisation,* 33: 33–44.

MARTIN, A. K., 1981, Evolution of the Agulhas Current and its paleoecological implications. *South African Journal of Sciences,* 77: 547–554.

MICHELSON, E. H. & DuBOIS, L., 1981, An isoenzyme marker possibly associated with the susceptibility of *Biomphalaria glabrata* populations to *Schistosoma mansoni. Acta Tropica,* 38: 419–426.

MULVEY, M. & VRIJENHOEK, R. C., 1981, Multiple paternity in the hermaphroditic snail, *Biomphalaria obstructa. Journal of Heredity,* 72: 308–312.

PARAENSE, W. L., 1955, Self- and cross-fertilisation in *Australorbis glabratus. Memórias do Instituto Oswaldo Cruz,* 53: 285–291.

ROLLINSON, D., 1979, The use of enzymes in taxonomy of *Bulinus. Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73: 601–602.

ROLLINSON, D. & SOUTHGATE, V. R., 1979, Enzyme analyses of *Bulinus africanus* group snails (Mollusca: Planorbidae) from Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73: 667–672.

SIMPSON, E. S. W., 1950, The geology and mineral resources of Mauritius. *Colonial Geology and Mineral Resources,* 1: 217–238.

STARMÜHLNER, F., 1976, Contribution to the knowledge of the fauna of running waters of Mauritius. *Bulletin of the Mauritius Institute,* 8: 105–128.

STARMÜHLNER, F., 1977, Contribution to the knowledge of the freshwater fauna of La Réunion (Mascerene). *Cahiers de l'Office de la Recherche Scientifiques et Technique, série Hydrobiologie,* 11: 239–250.

WRIGHT, C. A., 1957, Studies on the structure and taxonomy of *Bulinus jousseaumei* (Dautzenberg). *Bulletin of the British Museum (Natural History), Zoology,* 5: 1–28.

WRIGHT, C. A., 1971, *Bulinus* on Aldabra and the subfamily Bulininae in the Indian Ocean area. *Philosophical Transactions of the Royal Society of London* ser. B, 260: 299–313.

WRIGHT, C. A., ROLLINSON, D. & GOLL, P. H., 1979, Parasites in *Bulinus senegalensis* (Mollusca: Planorbidae) and their detection. *Parasitology,* 79: 95–105.

WRIGHT, C. A. & ROLLINSON, D., 1979, Analysis of enzymes in the *Bulinus africanus* group (Mollusca: Planorbidae) by isoelectric focusing. *Journal of Natural History,* 13: 263–273.

WRIGHT, C. A. & ROLLINSON, D., 1981, Analysis of enzymes in the *Bulinus tropicus/truncatus* complex (Mollusca: Planorbidae). *Journal of Natural History,* 15: 873–885.

WU, S.-K., 1972, Comparative studies on a polyploid series of the African snail genus *Bulinus* (Basommatophora: Planorbidae). *Malacological Review,* 5: 95–164.

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LES CARYOTYPES DE QUELQUES OSTREIDAE ET MYTILIDAE

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RÉSUMÉ

Les caryotypes d'*Ostrea edulis, Crassostrea gigas, Mytilus desolationis, Mytilus galloprovincialis* et *Mytilus edulis* ont été obtenus à partir des métaphases mitotiques de tissu branchial par des techniques de suspensions cellulaires.

Les résultats obtenus par Thiriot-Quiévreux & Ayraud (1982) sont précisés et complétés au cours de ce travail.

Ostrea edulis montre un caryotype (2n = 20) avec cinq classes de taille et est caractérisée par la présence de chromosomes métacentriques, submétacentriques et subtélocentriques. *Crassostrea gigas* a un caryotype (2n = 20) avec quatre classes de taille et des chromosomes métacentriques et submétacentriques. *Mytilus desolationis* a un caryotype (2n = 28) dont l'allure générale est semblable à ceux de *Mytilus galloprovincialis* et *Mytilus edulis*, à l'exception des paires 2 et 3. La différence structurale entre ces trois espèces s'observe au niveau de la paire 2 (constituée de chromosomes subtélocentriques chez *M. galloprovincialis*, métacentriques chez *M. edulis* et *M. desolationis*), et de la paire 3 (constituée de chromosomes subtélocentriques chez *M. galloprovincialis* et *M. edulis,* métacentriques ou submétacentriques chez *M. desolationis*).

Une discussion est abordée sur l'apport de la cytogénétique des Bivalves, du point de vue de la spéciation et de ses perspectives en aquaculture.

INTRODUCTION

Les travaux concernant la génétique des Bivalves se sont développés ces dernières années surtout d'un point de vue biochimique. L'étude des critères biochimiques, réalisée principalement par l'analyse d'électrophorégrammes des protéines, permet d'évaluer la structure génétique d'une population ou d'une espèce et d'envisager les variables écologiques du maintien du polymorphisme enzymatique. L'implication en aquaculture de l'étude du profil électrophorétique d'espèces commerciales suggère la possibilité de manipulation génétique pour améliorer la séléction et le taux de croissance (Singh & Zouros, 1981).

Du point de vue caryologique, jusqu'aux années 1970, les données sur les Bivalves se limitaient à la connaissance du nombre de chromosomes, puis les techniques utilisées, parfois adaptées au progrès des techniques de cytogénétique de Mammifères, permirent de préciser la morphologie des chromosomes et d'établir le caryotype des espèces. Depuis les revues de Patterson (1969) et Lubet (1976), plusieurs auteurs ont réalisé des études cytogénétiques sur les Bivalves. Le Tableau n^0 1 indique la liste des espèces étudiées. Dans les travaux mentionnés, d'une manière générale, les auteurs établissent les caryotypes en classant les chromosomes par ordre de taille, la morphologie des chromosomes (métacentriques, submétacentriques, télocentriques, subtélocentriques ou acrocentriques) est indiquée mais sans préciser leur position dans le caryotype, à l'exception des travaux de Rodriguez-Romero *et al.* (1978, 1979a,b,c) qui différencient les caryotypes des espèces de *Crassostrea* par la position des bivalents métacentriques ou submétacentriques.

Devant le peu d'information concernant les espèces comestibles, telles que *Ostrea edulis* Linné, 1758, *Crassostrea gigas* (Thunberg, 1793) et *Mytilus* spp., leur étude cytogénétique a été entreprise au moyen de techniques de suspensions cellulaires. En effet, la définition d'une espèce, quelque soit le critère utilisé pour l'identifier, doit comprendre la description du caryotype qui est de la plus haute importance pour la compréhension des mécanismes de spéciation et d'isolement reproductif. Prenons par exemple le cas de *Mytilus,* la systématique de ce genre est encore très confuse (Soot-Ryen, 1955). Les critères morphologiques sont sujets à des variations écologiques locales, des exemples d'hybridation ont été mis en évidence (Skibinski *et al.,* 1978; Gosling & Wilkins, 1981) et

le problème de la spéciation entre *Mytilus edulis* Linné, 1758 et *Mytilus galloprovincialis* Lamarck, 1819 reste ouvert (Gosling, 1982). L'étude cytogénétique de ces espèces apporte donc un critère supplémentaire indispensable.

Dans un article récent (Thiriot-Quiévreux & Ayraud, 1982), nous avons établi les caryotypes d'*Ostrea edulis, Crassostrea gigas, Mytilus galloprovincialis* et *Mytilus edulis*. Au cours de ce travail, des précisions seront apportées à ces résultats préliminaires et le

TABLEAU 1. Liste des études cytogénétiques récentes de Bivalves.

Espèces	Nombre diploide 2n	Auteurs	Techniques
Arcidae			
Barbatia virescens	28	Ieyama, 1975	fire-drying
Mytilidae			
Mytilus edulis	28	Ahmed & Sparks, 1970	squash
Mytilus californianus	28	Ahmed & Sparks, 1970	squash
Mytilus coruscus	28	Ieyama & Inaba, 1974	fire-drying
Isognomonidae			
Isognomon alatus	28	Wada, 1978	squash
Pteriidae			
Pinctada fucata	28	Wada, 1976	squash
Pinctada imbricata	28	Wada, 1978	squash
Pinnidae			
Pinna bicolor	32	Ieyama & Inaba, 1974	fire-drying
Pectinidae			
Pecten maximus	38	Beaumont & Gruffydd, 1974	squash
Pecten albicans	38	Ieyama, 1975	fire-drying
Chlamys distorta	38	Beaumont & Gruffydd, 1974	squash
Chlamys varia	38	Beaumont & Gruffydd, 1974	squash
Chlamys islandica	38	Beaumont & Gruffydd, 1974	squash
Placopecten magellanicus	38	Beaumont & Gruffydd, 1974	squash
Chlamys opercularis	26	Beaumont & Gruffydd, 1974	squash
Argopecten irradians	32	Wada, 1978	squash
Anomiidae			
Anomia chinensis	14	Ieyama & Inaba, 1974	fire-drying
Ostreidae			
Ostrea edulis	20	Longwell *et al.*, 1967	squash (sans fig.)
Ostrea lurida	20	Ahmed & Sparks, 1967	squash
Ostrea denselamellosa	20	Ieyama & Inaba, 1974	fire-drying
Ostrea circumpicta	20	Ieyama & Inaba, 1974	fire-drying
Crassostrea virginica	20	Longwell *et al.*, 1967	squash
		Rodriguez-Romero *et al.*, 1978, 1979a	air-drying
Crassostrea corteziensis	20	Rodriguez-Romero *et al.*, 1979b	air-drying
Crassostrea rhizophorae	20	Rodriguez-Romero *et al.*, 1979c	air-drying
Crassostrea gigas	20	Ahmed & Sparks, 1967	squash
		Ieyama & Inaba, 1974	fire-drying
Crassostrea belcheri	20	Ieyama & Inaba, 1974	fire-drying
Crassostrea ariakensis	20	Ieyama, 1975	fire-drying
Saccostrea echinata	20	Ieyama & Inaba, 1974	fire-drying
Saccostrea mordax	20	Ieyama & Inaba, 1974	fire-drying
Veneridae			
Ruditapes decussatus	38	Gerard, 1978	squash
Ruditapes philippinarum	38	Gerard, 1978	squash
Circe scripta	38	Ieyama, 1980	fire-drying
Paphia vernicosa	38	Ieyama, 1980	fire-drying
Irus mitis	38	Ieyama, 1980	fire-drying

caryotype de *Mytilus desolationis* Lamy, 1936 sera décrit. Enfin, une discussion sur les perspectives de la cytogénétique des Bivalves sera abordée.

MATERIEL ET METHODES

Ostrea edulis et *Crassostrea gigas* proviennent de naissains cultivés à Barfleur (côte nord, France).

Mytilus galloprovincialis a été récoltée dans des populations sauvages de Villefranche-sur-mer et de Brest.

Mytilus edulis provient de deux populations, l'une cultivée à Charron (côte ouest, France) et l'autre, sauvage, de Brest.

Mytilus desolationis est une espèce antarctique, qui a été ramenée en France par une mission des TAAF (Territoire des Terres Australes et Antarctiques Françaises) aux Iles Kerguelen en hiver 1982. Les moules de petite taille ont été gardées en élevage à Banuyls-sur-mer puis à Villefranche-sur-mer dans une eau de mer à 27‰, à la température de 8°C et nourries par des cultures de phytoplancton. Durant deux mois, leur croissance a été régulière puis l'élevage a périclité.

Les différentes étapes de la technique ont été indiquées en détail par Thiriot-Quiévreux & Ayraud (1982). Je résumerai ici les principaux points:

—bloquage des mitoses en métaphase par la colchicine à 0,005% dans de l'eau de mer pendant 16 heures,
—choc hypotonique d'une heure après dissection des branchies dans de l'eau de mer à 25%,
—fixation par 3 bains de 30 minutes dans l'alcool absolu (3 Vol.) et acide acétique (1 Vol.),

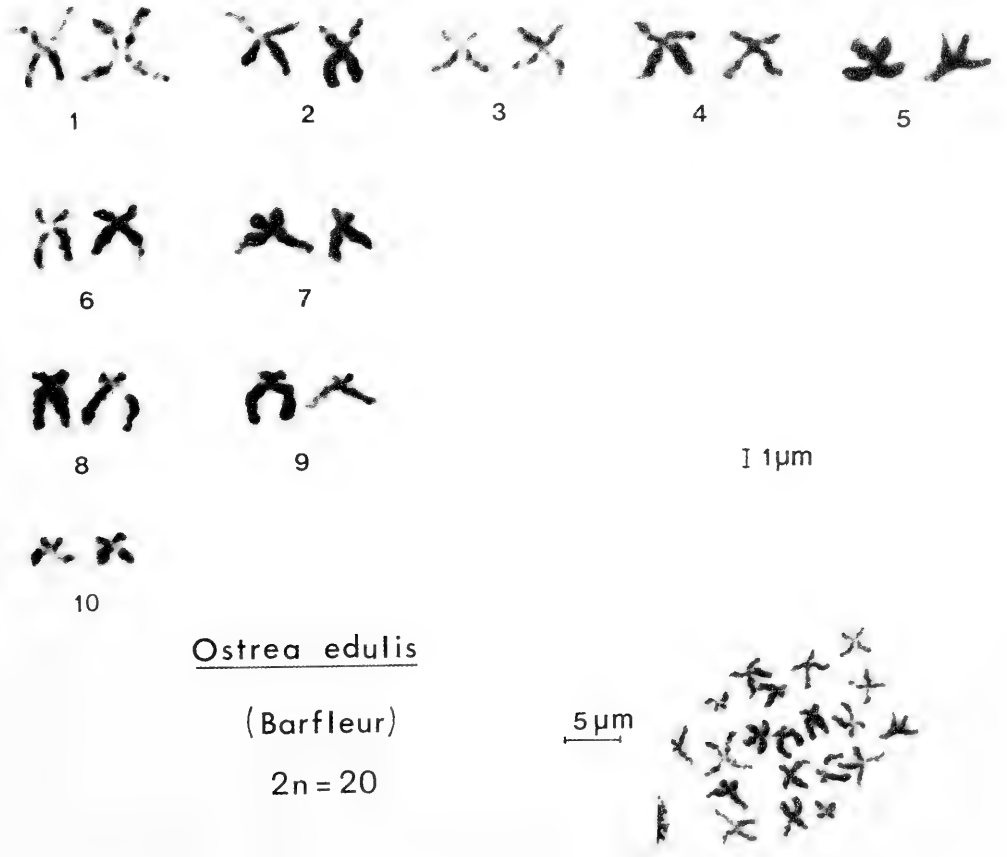

FIG.1. Métaphase mitotique et caryotype d'*Ostrea edulis* (technique standard au Giemsa).

—préparation des lames par dilacération du tissu branchial dans de l'eau acétifiée à 50% et projection de la suspension cellulaire sur des lames préchauffées à 44°C,
—coloration des lames pendant 10 minutes dans une solution de Giemsa à 4%, pH 6,7.

Certains animaux ont été placés dans une solution de 5-bromodéoxy-uridine (BRDU) à 50 µg/ml pendant quatre jours (*Ostrea*) ou six jours (*Crassostrea*), la colchicine à 0,005% étant additionnée à cette solution 12 heures avant la fin du traitement au BRDU.

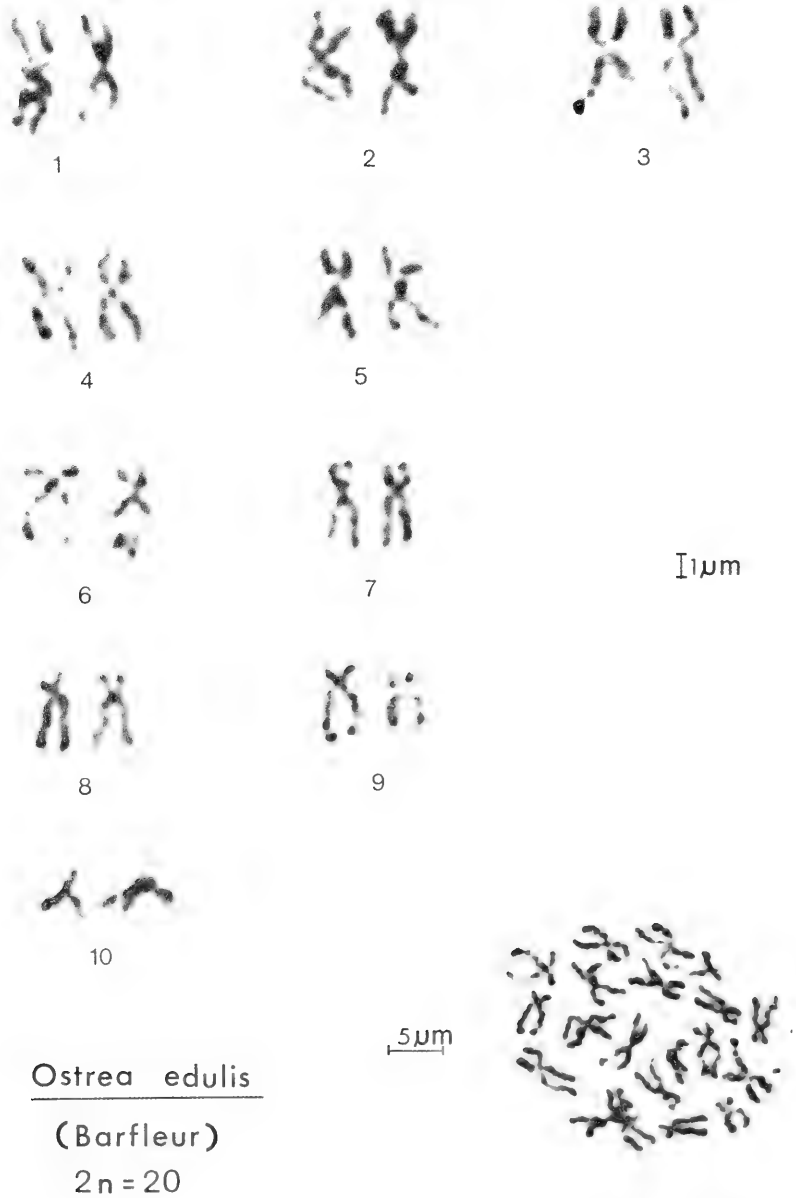

FIG. 2. Métaphase mitotique et caryotype d'*Ostrea edulis* (après traitement de quatre jours au 5-bromodéoxyuridine).

RESULTATS

Ostrea edulis

Avec l'utilisation de la technique standard au Giemsa, le caryotype (Fig. 1) montre quatre classes de taille. Le traitement au BRDU pendant quatre jours provoque un allongement des chromosomes qui permet de différencier 5 classes de taille (Fig. 2). Thiriot-Quiévreux & Ayraud (1982) avaient déjà pu mettre en évidence, avec un traitement de 48 heures au BRDU, cinq classes de taille; mais l'allongement des chromosomes est amélioré par un traitement prolongé et un début de marquage est nettement visible sur les chromosomes (paires 3 et 6 par exemple). Il est donc possible de confirmer la composition du caryotype d'*Ostrea edulis*:

Paires 1–3: chromosomes métacentriques; paires 4–5: chromosomes métacentriques de taille inférieure; paires 6–7: chromosomes submétacentriques; paires 8–9: chromosomes subtélocentriques; et paire 10: chromosomes métacentriques de petite taille.

Crassostrea gigas

Le traitement de six jours au BRDU met en évidence quatre classes de taille dans le caryotype de cette espèce (Fig. 3), les paires 2, 4 et 6 étant submétacentriques, les autres

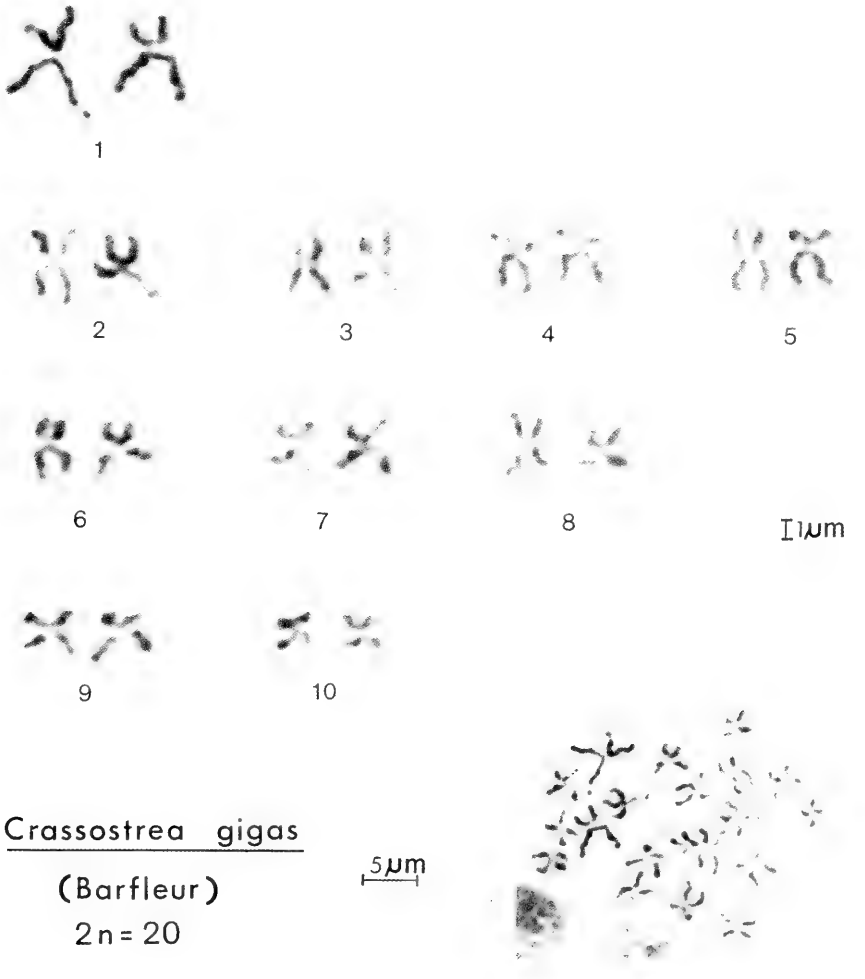

FIG. 3. Métaphase mitotique et caryotype de *Crassostrea gigas* (après traitement de six jours au 5-bromo-déoxyuridine).

métacentriques. Avec la technique standard au Giemsa (Thiriot-Quiévreux & Ayraud, 1982), la condensation des chromosomes n'avait pas permis d'établir des classes de taille.

Mytilus desolationis

Le caryotype de cette espèce, obtenu avec la technique standard au Giemsa, comprend 28 chromosomes. La paire 1 est constituée

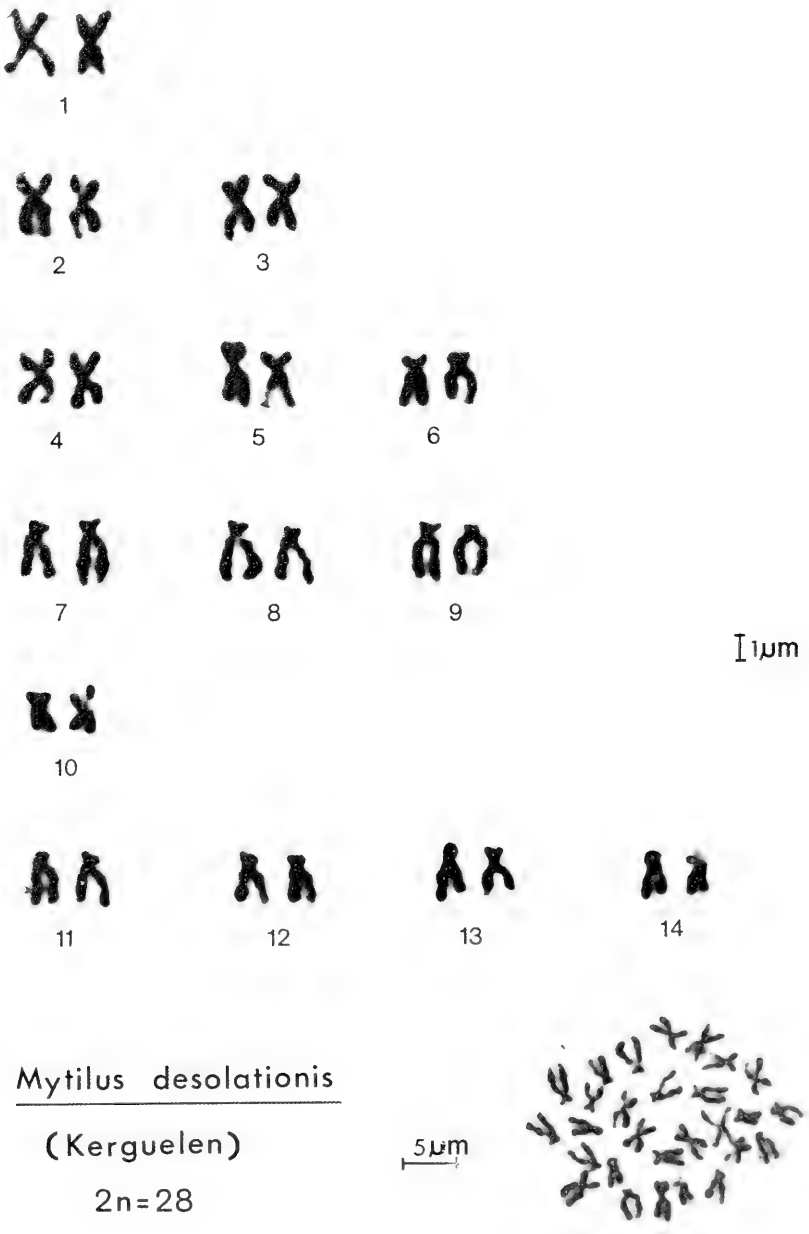

FIG. 4. Métaphase mitotique et caryotype de *Mytilus desolationis* (technique standard au Giemsa). Remarquer les paires 2 et 3 constituées de chromosomes métacentriques.

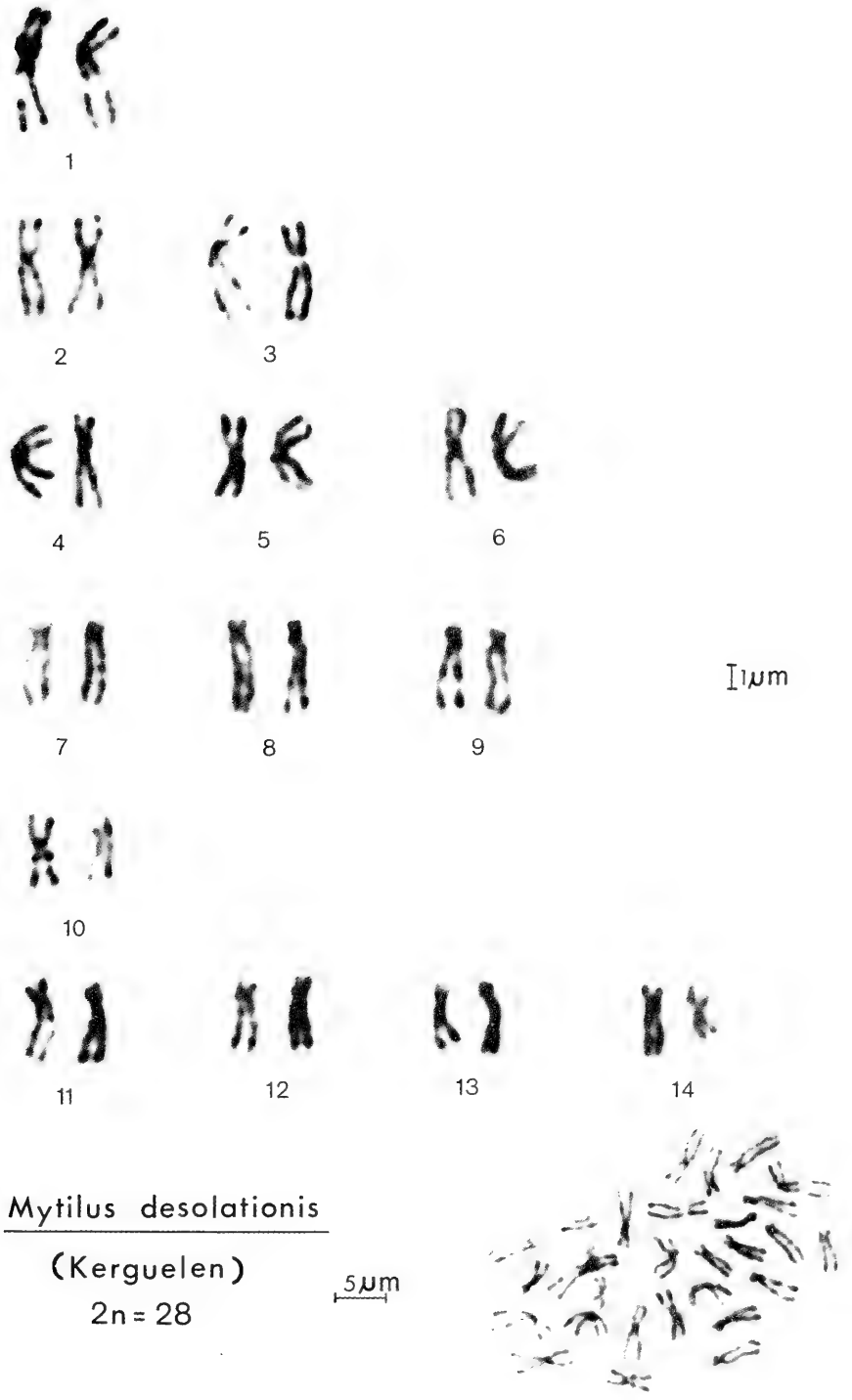

FIG. 5. Métaphase mitotique et caryotype de *Mytilus desolationis* (technique standard au Giemsa), exemple où la paire 3 est constituée de chromosomes submétacentriques.

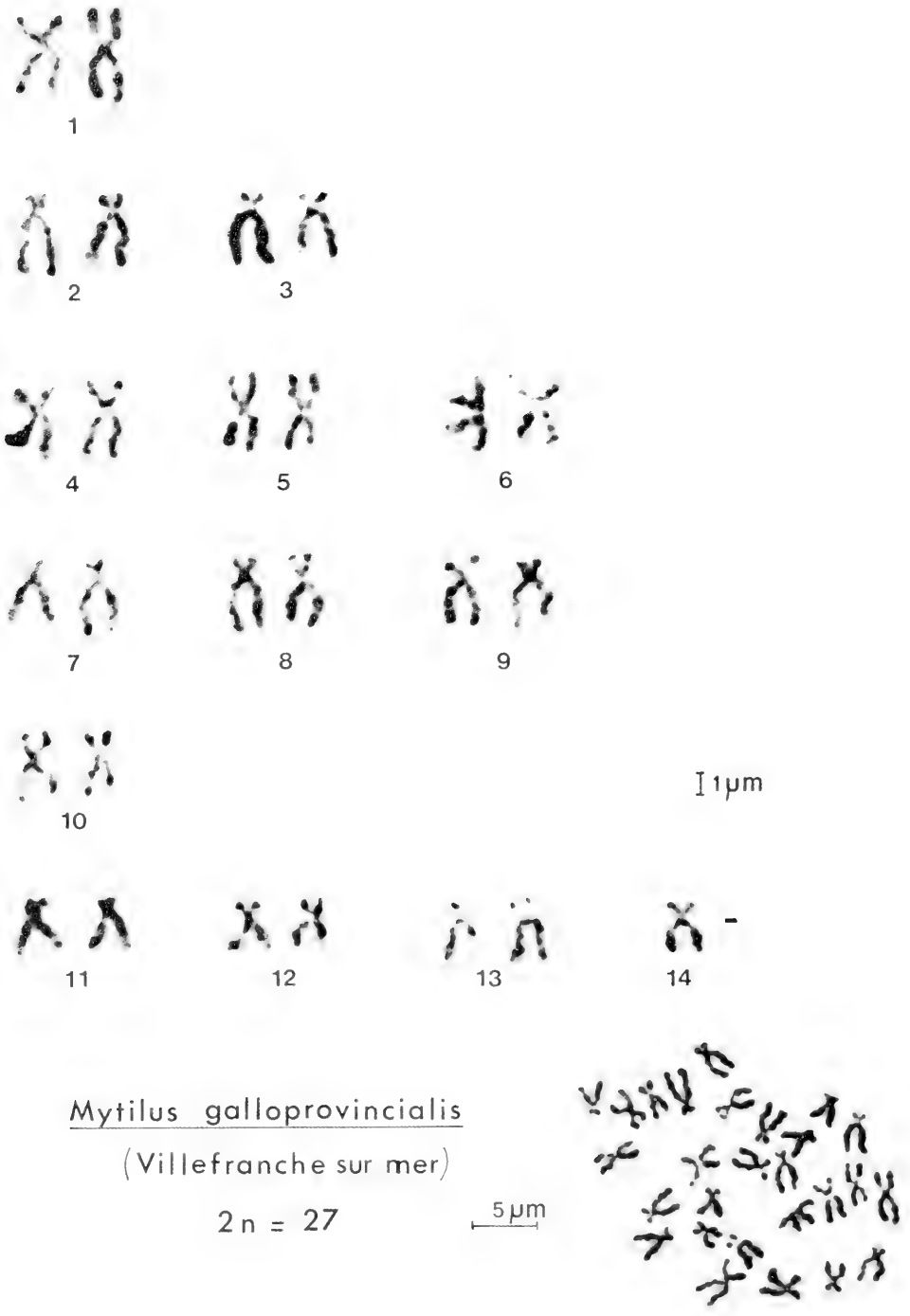

FIG. 6. Métaphase mitotique et caryotype de *Mytilus galloprovincialis* (exemple d'un nombre aneuploïde, technique standard au Giemsa). Remarquer les paires 2 et 3 constituées de chromosomes télocentriques.

de grands métacentriques, la paire 2 de métacentriques, la paire 3 de métacentriques (Fig. 4) ou de submétacentriques (Fig. 5), les paires 4 et 5 de métacentriques, la paire 6 de métacentriques (Fig. 5) ou submétacentriques (Fig. 4), les paires 7, 8 et 9 de subtélocentriques, la paire 10 de métacentriques et les paires 11 à 14 étant subtélocentriques au submétacentriques.

Sur 26 métaphases analysées correspondant à 13 animaux, 5 montraient une aneuploïdie.

Les paires 3 et 6 sont, selon les métaphases observées, métacentriques ou submétacentriques mais jamais subtélocentriques. Seule une analyse plus fine, avec des techniques de marquage des bandes "C" permettrait de préciser la position du centromère.

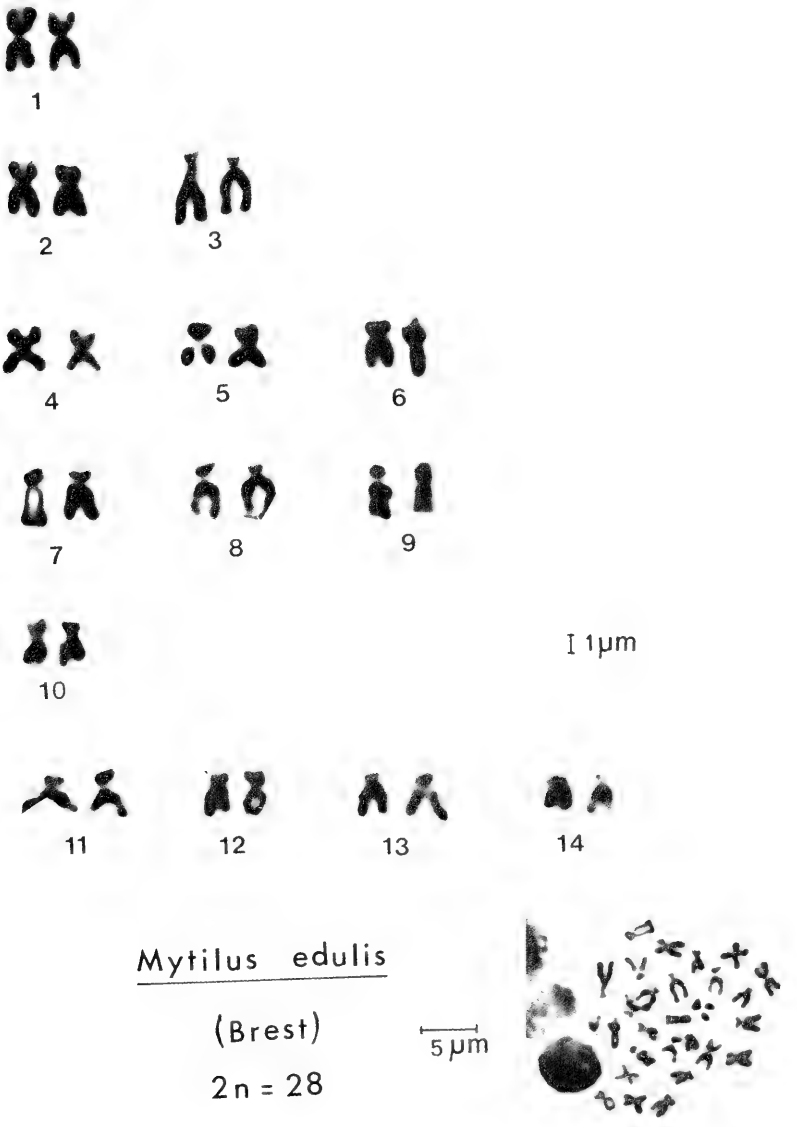

FIG. 7. Métaphase mitotique et caryotype de *Mytilus edulis* technique standard au Giemsa). Remarquer la paire 3 (chromosomes métacentriques) et la paire 2 (chromosomes télocentriques).

Cependant, d'une manière constante, la comparaison du caryotype de *Mytilus desolationis* avec ceux de *Mytilus galloprovincialis* et *Mytilus edulis* (Thiriot-Quiévreux & Ayraud, 1982) montre que l'allure générale du caryotype est identique chez les trois espèces, seules les paires 2 et 3 sont caractéristiques d'une espèce considérée. Chez *Mytilus galloprovincialis* (Fig. 6), elles sont toutes deux subtélocentriques. Chez *Mytilus edulis* (Fig. 7), la paire 2 est constituée de chromosomes métacentriques alors que la paire 3 est subtélocentrique. Chez *Mytilus desolationis,* elles sont métacentriques (ou parfois submétacentriques pour la paire 3). L'apparition d'inversions péricentriques pourrait expliquer l'évolution entre ces trois espèces. Mais seules les techniques de marquage pourraient préciser les différences morphologiques de ces bivalents.

DISCUSSION

Les techniques de suspension cellulaire ont apporté un net progrès dans la description des caryotypes. Il est actuellement possible de caractériser les différents types de chromosomes d'une espèce et de mettre en évidence des classes de taille. Mais il est important de souligner que ce n'est qu'un premier pas dans l'étude de la cytogénétique des Bivalves. Des précisions et des améliorations restent à envisager.

Nous avons vu qu'un traitement prolongé au BRDU provoque un net allongement des chromosomes en début de marquage. La mise en évidence simultanée des bandes et des chromatides soeurs (Fonatsch, 1979) est l'étape suivante de cette étude. En effet, les échanges entre chromatides soeurs semblent exister naturellement mais augmentent sous l'action d'agents chimiques, physiques, polluants ou mutagènes et peuvent donc être utilisés comme test de mutagénèse.

La technique standard au Giemsa permet d'établir un caryotype mais nos travaux en cours, avec des techniques de marquage, sont nécessaires afin de préciser les différences structurales existant entre les paires de chromosomes comme c'est le cas pour *Mytilus.* Nous avons suggéré que des inversions péricentriques au niveau des paires 2 et 3 pouvaient traduire l'évolution entre les trois espèces de *Mytilus* étudiées. En effet, les inversions péricentriques sont caractéristiques de l'évolution entre espèces (Dutrillaux, 1979; Wright, 1982), mais ces inversions sont toujours indiquées au niveau des bandes. Ce sera l'objet de notre étude ultérieure chez *Mytilus.*

Les divers modes de variation du caryotype des populations de *Mytilus galloprovincialis* et *Mytilus edulis,* récoltées lorsque ces espèces coexistent et montrent des formes hybrides, apporteraient des renseignements complémentaires au problème de la spéciation entre ces deux espèces, actuellement controversée (Gosling, 1982). De plus, l'étude des caryotypes d'hybrides des générations F_1 et F_2, obtenus en élevages, serait également un argument pour résoudre leur taxonomie.

Le caryotype de *Mytilus desolationis* semble indiquer la présence d'une espèce propre aux Kerguelen, différente tout au moins cytogénétiquement de *Mytilus edulis.* Seule une étude comparée des autres espèces antarctiques de *Mytilus* permettrait d'envisager l'origine de cette espèce, dont les populations constituent de véritables Moulières (Arnaud, 1971).

L'aneuploïdie, déjà notée par Ahmed & Sparks (1970) chez *Mytilus edulis* et *Mytilus californianus,* se retrouve chez certaines espèces. Thiriot-Quiévreux & Ayraud (1982) indiquent, pour *Ostrea edulis* une aneuploïdie entre 5 et 10% des métaphases analysées; des cas d'aneuploïdie ont été également observés chez *Mytilus galloprovincialis* et *Mytilus edulis* mais sans en préciser leur pourcentage. Pour *Mytilus desolationis,* 1/5 des métaphases observées sont aneuploïdes. Il faut remarquer que les métaphases aneuploïdes et normales coexistent dans un même animal. Par contre, pour *Crassostrea gigas,* sur 20 métaphases analysées, toutes étaient normales.

La signification de cette aneuploïdie reste à élucider. Une première explication pourrait correspondre à des aléas de la technique, mais le cas de *Crassostrea gigas,* où toutes les métaphases étaient normales, ne permet pas de généraliser les pertes de chromosomes dues aux manipulations. Seule une étude statistique, d'un plus grande nombre de métaphases, sur toutes les espèces étudiées, pourrait préciser la réalité de cette aneuploïdie. Une deuxième hypothèse, émise par Zouros (communication personnelle) serait une tentative d'expliquer la déficience en hétérozygotie chez les Bivalves (Zouros & Foltz, 1984). S'il existe un certain nombre d'animaux présentant un nombre de chromosomes inférieur à 2n, le nombre d'allèles diminuerait et cela se traduirait par une augmentation apparente d'homozygotes. Le

problème est que, jusqu'à présent, les observations ont montré la coexistence de métaphases normales et aneuploïdes dans un même animal. Ces questions laissent entrevoir l'intérêt d'une étude ultérieure.

La cytogénétique des Bivalves est actuellement une discipline jeune. Son apport à la génétique des Bivalves, du point de vue de la spéciation, est indéniable. Son implication peut également être envisagée en aquaculture. Des expériences d'induction de polyploïdie (Stanley *et al.*, 1981, 1982) ont été tentées pour améliorer le poids des animaux, la polyploïdie engendrant la stérilité, les animaux produit ainsi devraient avoir un poids supérieur (leur réserve n'étant pas utilisée pour la reproduction). Ces expériences ont été tentées avec succès chez les Poissons (Allen & Stanley, 1978).

La corrélation entre l'hétérozygotie et le taux de croissance, mise en évidence par des études biochimiques (Koehn *et al.*, 1976; Zouros *et al.*, 1980; Beaumont, 1982) montre l'intérêt de la recherche d'hétérozygotes pour un meilleur rendement de croissance. La recherche de marqueurs génétiques, tant biochimiques que cytogénétiques est un pas considérable pour la sélection des géniteurs.

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Je remercie la Société SATMAR à Barfleur pour ses envois de naissains d'*Ostrea edulis* et de *Crassostrea gigas* ainsi que la Direction des Laboratoires Scientifiques des TAAF (Territoires des Terres Australes et Antarctiques Françaises) et Mr. J. Soyer, responsable du programme "Génétique des populations d'Invertébrés marins benthiques (Benthos, KER)" qui ont permis la récolte de *Mytilus desolationis* et son élevage jusqu'au Laboratoire de Villefranche-sur-mer.

Mr. le Professeur Ayraud (Laboratoire de Cytogénétique de la Faculté de Médecine de Nice) m'a prodigué ses conseils pour l'interprétation des caryotypes et m'a permis l'utilisation des équipements de microscopie de son laboratoire pour la réalisation des microphotographies.

LITTÉRATURE CITÉE

AHMED, M. & SPARKS, A. K., 1967, A preliminary study of chromosomes of two species of oysters (*Ostrea lurida* and *Crassostrea gigas*). *Journal of the Fisheries Research Board of Canada*, 24: 2155–2159.

AHMED, M. & SPARKS, A. K., 1970, Chromosome number, structure and autosomal polymorphism in the marine mussels, *Mytilus edulis* and *Mytilus californianus*. *Biological Bulletin*, 136: 1–13.

ALLEN, S. K. & STANLEY, J. G., 1978, Reproductive sterility in polyploid brook trout, *Salvelinus frontinalis*. *Transactions of American Fisheries Society*, 107: 474–478.

ARNAUD, P., 1971, Les Moulières à *Mytilus* et *Aulacomya* des Iles Kerguelen (sud de l'Océan Indien). Les "moulières de seuil" et leur intérêt possible pour l'aquaculture des Pélécypodes. *Comptes Rendus des Séances de l'Académie des Sciences [Paris]*, 272: 1423–1425.

BEAUMONT, A. R., 1982, Variations in heterozygoty at two loci between year classes of a population of *Chlamys opercularis* (L.) from a Scottish sealoch. *Marine Biology Letters*, 3: 25–33.

BEAUMONT, A. R. & GRUFFYDD, L. L., 1974, Studies on the chromosomes of the scallop *Pecten maximus* (L.) and related species. *Journal of the Marine Biological Association of the United Kingdom*, 54: 713–718.

DUTRILLAUX, B., 1979, Chromosomal evolution in primates. Tentative phylogeny from *Microcebus murinus* (prosimian) to man. *Human Genetics*, 48: 251–314.

FONATSCH, C., 1979, A technique for simultaneous demonstration of G bands and sister chromatid exchanges. *Cytogenetics and Cell Genetics*, 23: 144–146.

GERARD, A., 1978, Etude des garnitures chromosomiques de deux Veneridae: *Ruditapes decussatus* (L.) et *Ruditapes philippinarum* (Adams et Reeve). *Haliotis*, 9: 69–71.

GOSLING, E., 1984, The systematic status of *Mytilus galloprovincialis* in Western Europe. *Second International Symposium on Molluscan Genetics, New Orleans; Malacologia*, 25: 551–568.

GOSLING, E. & WILKINS, N. P., 1981, Ecological genetics of the mussels *Mytilus edulis* and *Mytilus galloprovincialis* on Irish coasts. *Marine Ecology Progress Series*, 4: 221–227.

IEYAMA, H., 1975, Chromosome numbers of three species in three families of Pteriomorphia (Bivalvia). *Venus; Japanese Journal of Malacology*, 34: 26–32.

IEYAMA, H., 1980, Studies on the chromosomes in three species of the Veneridae (Bivalvia, Heterodonta). *Venus; Japanese Journal of Malacology*, 39: 49–55.

IEYAMA, H. & INABA, A., 1974, Chromosome numbers of ten species in four families of Pteriomorphia (Bivalvia). *Venus; Japanese Journal of Malacology*, 33: 129–137.

KOEHN, R. K., MILKMAN, R. & MITTON, J. B., 1976, Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel *Mytilus edulis*. *Evolution*, 30: 2–32.

LONGWELL, A. C., STILES, S. S. & SMITH, D. G., 1967, Chromosome complement of the Amer-

ican oyster *Crassostrea virginica* as seen in meiotic and cleaving eggs. *Canadian Journal of Genetics and Cytology,* 9: 845–856.

LUBET, P., 1976, L'espèce chez les lamellibranches marins. *Mémoires de la Société Zoologique de France,* 368: 341–368.

PATTERSON, C. M., 1969, Chromosomes of molluscs. *Proceedings 2nd Symposium on Mollusca, Marine Biological Association of India:* 635–686.

RODRIGUEZ-ROMERO, F., LAGUARDA-FIGUERAS, A., URIBE-ALCOCER, M. & ROJAS-LARA, M. L., 1979a. Distribution of "G" bands in the karyotype of *Crassostrea virginica. Venus; Japanese Journal of Malacology,* 38: 180–184.

RODRIGUEZ-ROMERO, F., URIBE-ALCOCER, M. & LAGUARDA-FIGUERAS, A., 1978, Cytogenetic study of an oyster population of the species *Crassostrea virginica* Gmelin from the coasts of Tabasco, Mexico. *Venus; Japanese Journal of Malacology,* 37: 83–86.

RODRIGUEZ-ROMERO, F., URIBE-ALCOCER, M. & LAGUARDA-FIGUERAS, A., 1979b, The karyotype of *Crassostrea corteziensis* Hertlein (Mollusca: Ostreidae). *An. Centro Ciencias del Mar y Limnologia, Universidad Nacional Autonoma de Mexico,* 6(1): 15–18.

RODRIGUEZ-ROMERO, F., URIBE-ALCOCER, M., LAGUARDA-FIGUERAS, A. & DIUPOTEX-CHONG, M. E., 1979c, The karyotypes of *Crassostrea rhizophorae* (Guilding, 1828). *Venus; Japanese Journal of Malacology,* 38: 135–140.

SINGH, S. M. & ZOUROS, E., 1981, Genetics of growth rate in oysters and its implication for aquaculture. *Canadian Journal of Genetics and Cytology,* 23: 119–130.

SKIBINSKI, D. O. F., AHMAD, M. & BEARDMORE, J. A., 1978, Genetic evidence for naturally occurring hybrids between *Mytilus edulis* and *Mytilus galloprovincialis. Evolution,* 32: 354–364.

SOOT-RYEN, T., 1955, A report on the family Mytilidae (Pelecypoda). *Allan Hancock Pacific Expeditions,* 20(1): 1–154.

STANLEY, J. G., ALLEN, S. K. & HIDU, H., 1981, Polyploidy induced in the american oyster, *Crassostrea virginica,* with cytochalasin B. *Aquaculture,* 23: 1–10.

STANLEY, J. G., ALLEN, S. K. & HIDU, H., 1982, Growth, fertility and heterozygoty in triploid shellfish. *International Symposium on Genetics in Aquaculture,* Galway, 1982.

THIRIOT-QUIÉVREUX, C. & AYRAUD, N., 1982, Les caryotypes de quelques espèces de Bivalves et de Gastéropodes marins. *Marine Biology,* 70: 165–172.

WADA, K., 1976, Number and gross morphology of chromosomes in the pearl oyster, *Pinctada fucata,* collected from two regions of Japan. *Venus; Japanese Journal of Malacology,* 35: 9–14.

WADA, K., 1978, Chromosome karyotypes of three bivalves: the oysters, *Isognomon alatus* and *Pinctada imbricata* and the bay scallop, *Argopecten irradians. Biological Bulletin,* 155: 235–245.

WRIGHT, S., 1982, Character change speciation, and the higher taxa. *Evolution,* 36: 427–443.

ZOUROS, E. & FOLTZ, D. W., 1984, Possible explanations of heterozygote deficiency in bivalve molluscs. *Second International Symposium on Molluscan Genetics, New Orleans; Malacologia,* 25: 583–591.

ZOUROS, E., SINGH, S. M. & MILES, H. E., 1980, Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution,* 34: 856–867.

KARYOTYPES OF SOME OSTREIDAE AND MYTILIDAE (BIVALVIA)

Catherine Thiriot-Quiévreux

ABSTRACT

Karyotypes of *Ostrea edulis, Crassostrea gigas, Mytilus desolationis, Mytilus galloprovincialis* and *Mytilus edulis* were obtained from mitotic metaphases of branchial tissue with the cellular suspension technique.

Additional results from those published by Thiriot-Quiévreux and Ayraud (1982) are discussed in the present paper.

Ostrea edulis shows a karyotype (2n = 20) with four size classes of metacentric, submetacentric and subtelocentric chromosomes. *Crassostrea gigas* has a karyotype (2n = 20) with 4 size classes of metacentric and submetacentric chromosomes. *Mytilus desolationis* has a karyotype (2n = 28) with the same general pattern as *Mytilus galloprovincialis* and *Mytilus edulis,* except for pairs 2 and 3. The differences between these three species are located on pair 2: subtelocentric in *M. galloprovincialis,* metacentric in *M. edulis* and *M. desolationis,* and in pair 3: subtelocentric in *M. galloprovincialis* and *M. edulis,* metacentric or submetacentric in *M. desolationis.*

The contribution of cytogenetics to the genetics of the Bivalvia is discussed from the speciation point of view and with regard to the implications in aquaculture.

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SPECIES COHESIVENESS AND GENETIC CONTROL OF SHELL COLOR AND FORM IN *THAIS EMARGINATA* (PROSOBRANCHIA, MURICACEA): PRELIMINARY RESULTS

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ABSTRACT

Thais (or *Nucella*) *emarginata* has one of the largest geographical ranges of rocky-intertidal, prosobranch gastropods of the northeastern Pacific, nearly 35° latitude. *T. emarginata* has direct development from benthic egg capsules with no pelagic larvae. Throughout its latitudinal range, *T. emarginata* is restricted to the mid- and upper-intertidal zone of rocky shores, and is generally restricted to areas with intermediate to high wave exposure or moderate currents. Consequently, expanses of deep water, sand beaches and quiet estuaries or inlets all act to inhibit gene flow. In addition, lateral movements along rocky shores are not very great; mean distances moved by marked individuals over a 12 month period were less than 5 m.

Pairwise crosses of *T. emarginata* from southeast Alaska (U.S.A.) and Vancouver Island (Canada) revealed that in spite of restricted gene flow, individuals from these distant populations (circa 1500 km) were capable of producing F1 offspring that grew to adult size, mated and produced egg capsules. F2 viability remains to be determined. Females from Alaska produced fewer egg capsules (avg. <50%) in the laboratory than those from Vancouver Island, but there were no differences between within- or between-population crosses. No consistent differences in juvenile survivorship were noted among crosses of each type.

T. emarginata also exhibits variation in shell color, banding and sculpture. Shell-morph frequencies of F1 offspring suggest that: 1) there are at least three discrete color alleles (black, orange, white), 2) the color black is dominant to orange and white,[1] 3) banding assorts independently of color, 4) the capacity to produce spiral sculpture may be controlled by a single switch gene or a block of tightly linked genes, and 5) spiral sculpture assorts independently of color.

Key words: *Thais*; shell; variation; genetics; breeding; marine; dispersal; geographic range; speciation.

INTRODUCTION

It is a common view that genetic divergence results from reduced or interrupted gene flow among populations, either due to genetic drift or to selection (Mayr, 1963; Endler, 1977; Lessios, 1981). If of sufficient magnitude, such divergence may ultimately lead to speciation, a process which remains poorly understood (Ayala, 1975; Avise, 1976; Bush, 1982). One approach to the study of speciation is to examine the degree of genetic divergence among populations of a geographically wide-ranging species via breeding experiments. Such experiments provide information about offspring viability as a function of geographic separation of parents and may also provide information about the nature

[1]NOTE ADDED SEPTEMBER 1983: F2 progeny have revealed a more complicated set of dominance relationships for color than indicated below. In Alaskan populations, the color black is clearly dominant to brown, and both are dominant to orange; F2 phenotype frequencies indicate a simple, single-locus, three-allele basis of color variation in lineage 16 (now 80–16). In Vancouver Island populations [e.g. lineages 17 and 18 (now 80–17 and 80–18)], however, either more alleles are present, more loci are involved or epistatic effects on expression of color are greater since the color orange exhibits considerable variation in intensity of expression. Of greater significance, orange can be completely dominant to black, although black exhibits weak and variable penetrance in some crosses, and black so far exhibits no evidence of dominance over orange. At the present time, I am not certain whether the color loci in Alaskan and Vancouver Island populations are the same; however, differences clearly exist in the phenotypic dominance hierarchy among color alleles between these distant populations. Thus, the interpretation presented below that regulation of color expression may have broken down in between-population crosses, appears to be supported.

of differences that lead to speciation (Ayala, 1975; Marcus, 1980).

I report here the results of some breeding experiments with individuals from geographically distant populations of the rocky-intertidal gastropod, *Thais* (or *Nucella*) *emarginata* (Deshayes, 1839). In addition to information on genetic divergence, these crosses permit a preliminary interpretation of the genetic basis of variation in color, banding and sculpture in a marine, prosobranch gastropod. Data of these types have been reported for few such gastropods (*Littorina picta* [Struhsaker, 1968], *Urosalpinx cinerea* [Cole, 1975], and *Littorina saxatilis* [Newkirk & Doyle, 1975]).

METHODS

Geographic ranges of prosobranch gastropods that occur in the rocky intertidal zone of the central and northern Pacific coast of North America were obtained from Abbott (1974) and McLean (1966). Species that were largely or strictly intertidal were distinguished from those that were predominantly subtidal.

Data on vertical distribution of *Thais* in the intertidal zone and lateral distribution along a wave-exposed gradient were obtained in Torch Bay, Alaska (58°20′ N, 136°50′ W), a deep, 6 km long fjord that opens on the Gulf of Alaska. Six replicate 0.1 m^2 quadrat samples were taken at 0.5 m or 1.0 m intervals along two permanent vertical transects in an area of intermediate wave exposure in 1974, 1978 and 1979. Counts were made of all three species of *Thais* that occurred at these sites [*T. canaliculata* (Duclos, 1832), *T. emarginata* and *T. lamellosa* (Gmelin, 1791)]. The distribution along a wave exposure gradient was determined by inspecting the shores of Torch Bay in June 1978 as indicated in Fig. 3, and recording the presence or absence of *T. emarginata*.

Movements of *T. emarginata* were determined by recording the distance moved by marked individuals from their point of release. Vertical movements of *T. emarginata* were compared to those of the lower shore *T. canaliculata* at Iceberg Point, Lopez Island, Washington (U.S.A.) in July, 1974. Individuals of both species were collected and returned to the laboratory where they were marked by writing numbers on their shells with india ink and coating them with a clear cement (Dekophane, Rona Pearl Corp., Bayonne, New Jersey). The day after collection, 50 marked individuals of each species were released at two positions on the shore: high, at the base of the *Balanus glandula* refuge zone and low, in the lower third of the *Semibalanus cariosus* zone. Two weeks after release, the positions of all marked snails that could be located were noted. Lateral movements along the shore were measured on marked *T. emarginata* at two sites in Torch Bay, Alaska from June 1978 to June 1979 relative to their point of release in 1978.

Breeding experiments were initiated in September 1980 with *T. emarginata* collected from Torch Bay, Alaska and Wizard Rock, near the Bamfield Marine Station (Bamfield, British Columbia, Canada; 48°53′ N, 125°10′ W). Immature snails were sexed by anaesthetizing them overnight in a $MgCl_2$ solution ($MgCl_2$ mixed with tap water to a specific gravity the same as seawater at approximately 32‰ then diluted 1:3 with seawater). The feet of the relaxed animals were pulled gently out of the aperture until it was possible to determine the presence or absence of a penis behind the right tentacle. As nearly all *T. emarginata* had a penis when small, it was necessary to rank individuals by penis size; those with a large penis were considered males and those with a small penis were considered females. To date, sexing errors have occurred in fewer than 10% of the crosses as verified by penis size when mature. Snail sizes were measured as shell length to the nearest 0.1 mm with vernier calipers.

Pairs of sexed, immature snails were held in plastic freezer containers with sides of 3 mm mesh VEXAR (Dupont) plastic screen and supplied with small stones covered with the barnacle *Balanus glandula,* one of their preferred prey (Palmer, 1980). The cages were immersed in aquaria with continuously running sea water at the Bamfield Marine Station and barnacles were replenished after they were consumed. Egg capsules were allowed to remain in these cages until close to hatching (10 to 14 weeks) at which point they were transferred to freezer containers with NITEX screen sides (335 μm mesh). Newly hatched snails were supplied with recently metamorphosed *B. glandula* on small stones collected from the field. When large enough (>3 mm), juveniles were transferred to coarser mesh, freezer-container cages where they were grown to adult size on larger *B.*

glandula. As adult coloration became discernible (8–10 mm shell length), the F1 progeny were photographed at approximately 8 week intervals to record the pattern of color maturation.

RESULTS

Of species of prosobranch gastropods that occur in the rocky intertidal, only three of 91 have latitudinal ranges as great or greater than *Thais emarginata* (Fig. 1). Of strictly rocky-intertidal prosobranchs, only one of 35 species (the limpet *Collisella pelta*) has a latitudinal range as great as *T. emarginata* along the Pacific coast of North America (Fig. 1).

The vertical transects in Torch Bay revealed that even though the distribution of *Thais emarginata* changed rather dramatically over time (Fig. 2), this species rarely occurred below +1.0 m above MLLW. In Washington, marked individuals released at or below this point actively crawled upshore while individuals released within this zone tended to remain there (Table 1).

The shore survey of Torch Bay revealed that *T. emarginata* did not occur in the protected portions of the bay and occurred infrequently on the most exposed headlands (Fig. 3). In addition, in Torch Bay, *T. emarginata* was rarely found on cobble beaches unless the snails were immediately adjacent to a stretch of bedrock shore. Lateral movements of marked animals along continuous bedrock at two sites on the north-central shore of Torch Bay varied as a function of wave exposure (Fig. 4). At neither site, however, were individuals recovered that had moved more than 10 m over 12 months. I have observed movements of greater distances for *T. emarginata* in the San Juan Islands, Washington (U.S.A.), but they were not common; only 6 of 92 marked animals recovered more than 10 m over 2 months along the shore of Deadman Island (Palmer, unpublished).

Egg capsule production varied widely among pairs (Table 2). In general, Alaskan females produced fewer than half the number of egg capsules produced by those from Vancouver Island; however there were no consistent differences in egg capsule production or juvenile survivorship between within- and between-population crosses (Table 2).

Tables 3–5 present parental and offspring phenotypes from within- and between-

TABLE 1. Mean distances moved following reciprocal vertical transplants of *Thais emarginata* and *T. canaliculata* at Iceburg Point, Lopez Island, Washington (U.S.A.) in July 1974. Distances were measured along the rock surface relative to the release point. Fifty animals of each species were released at each location. The upper release point was at the base of the *Balanus glandula* refuge zone, the lower release point was in the lower third of the *Semibalanus cariosus* zone. Tabled values are means ± one standard error. P = probability value for two-tailed t-test.

Species	N	Mean	P
Distance moved down from upper release point (m)			
T. emarginata	12	0.44(0.147)	0.19
T. canaliculata	4	0.87(0.335)	
Distance moved up from lower release point (m)			
T. emarginata	25	4.05(0.885)	<0.001
T. canaliculata	17	1.19(0.505)	

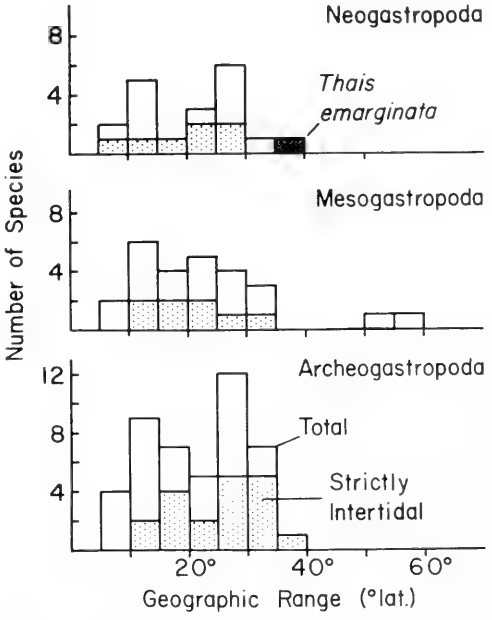

FIG. 1. Frequency distribution of geographic ranges (degrees latitude) of prosobranch gastropods recorded from the rocky intertidal zone of the central and northern Pacific coast of North America. Ranges of archeogastropods from McLean (1966), all others from Abbott (1974). Stippled bars represent species restricted largely to the intertidal zone.

TABLE 2. Egg capsule production rates and survivorship of juveniles of *Thais emarginata* from within- and between-population crosses of animals collected from Torch Bay, Alaska (U.S.A.) and Bamfield, British Columbia (Canada). Tabled values include the total number of egg capsules produced by pairs in the laboratory from Sept. 2, 1980 to Dec. 28, 1981, and the number of juveniles surviving per capsule on Dec. 29, 1981.

		Female				
	Torch Bay			Bamfield		
Male	Cross	Caps.	Juv.	Cross	Caps.	Juv.
Torch Bay	15	44	0.32	1	110	0.33
	16	50	0.26	2	101	0.84
				11	124	0.02
				12	58	0.00
	Means	47.0	0.29		98.3	0.30
Bamfield	3	59	0.25	17	95	0.48
	4	47	0.49	18	102	0.26
	13	52	0.46			
	14	19	0.14			
	Means	44.3	0.34		98.5	0.37

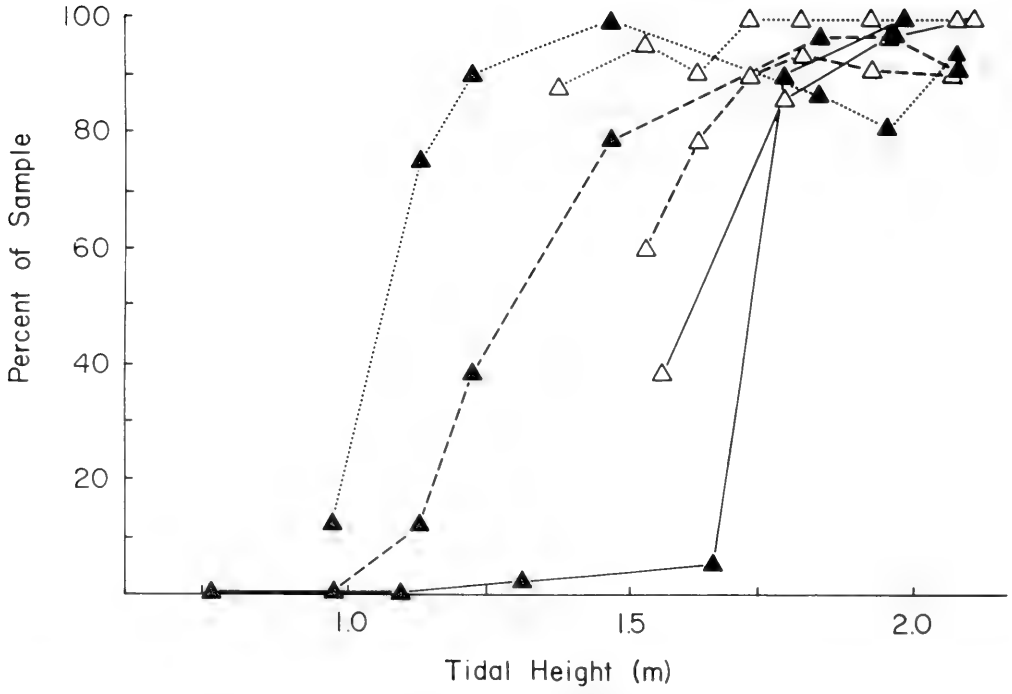

FIG. 2. Vertical distribution of *Thais emarginata* in Torch Bay, Alaska (U.S.A.) for three different years: 1974 (solid lines), 1978 (dashed lines) and 1979 (dotted lines). Solid symbols and open symbols represent data from two separate transects. Tidal height was measured in meters above MLLW. Mean maximum tidal range in the vicinity of Torch Bay is 3.1 m (Anonymous, 1982).

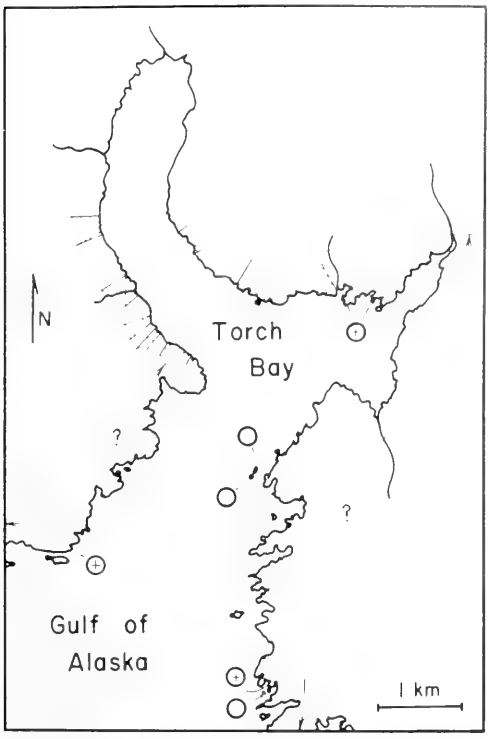

FIG. 3. Lateral distribution of *Thais emarginata* along the shore of Torch Bay, Alaska (U.S.A.) in June 1978. Stippled areas indicate continuous populations. '+' and '−' indicate presence or absence at isolated sites. Regions of the shore not surveyed are indicated by question marks.

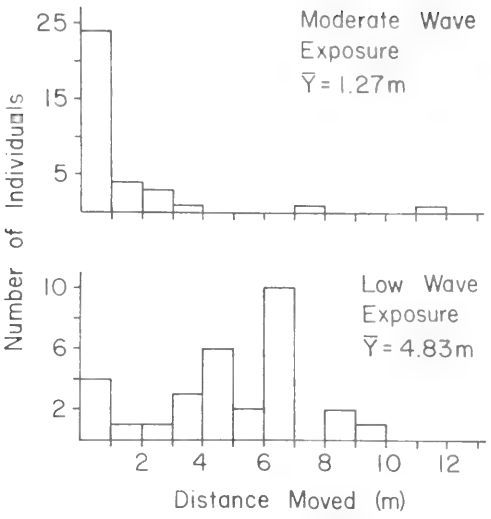

FIG. 4. Frequency distribution of distances moved by marked *Thais emarginata* at two sites of differing wave exposure in Torch Bay, Alaska (U.S.A.) over 12 months, from June 1978 to June 1979. At both sites the shore was searched for 15 or more meters in both directions from the site of release.

population crosses. Three discrete color morphs could be recognized clearly: black, orange and white. Variable shades of brown, orange-brown, grey-orange and grey also existed but were difficult to score among broods reliably. Black appeared dominant to orange (crosses 1 and 16) and white appeared to be recessive to black and orange (cross 15, Table 3), although there was some evidence of blending. While most crosses yielded an intermediate range of sculptural development, one (cross 18, Table 5) yielded two distinct phenotypes: strong spiral sculpture and smooth. In this cross, sculpture assorted independently of the colors orange and black (Table 6). Banding also assorted independently of color (cross 17, Table 6). Finally, the offspring of cross 4 exhibited a rather dramatic linkage between growth rate (or possibly time of hatching) and shell color: after 8 months of growth, the seven brown-banded individuals were significantly larger (mean shell length = 21.1 mm, SE = 1.02) than the 13 orange-brown individuals (mean = 13.0 mm, SE = 0.57; $P < 0.001$, t-test).

DISCUSSION

Geographic distribution

The geographic range of *Thais emarginata* is clearly large relative to other prosobranch gastropods that occur in the rocky intertidal (Fig. 1). This species thus appears capable of persisting under a wide range of physiological and ecological conditions. Its range includes three 'marine-climate' and four 'wave-climate' regions along the Pacific coast of North America (Hayden & Dolan, 1976), and spans three biogeographic provinces described by Valentine (1966) based on molluscs and four faunal associations recognized by Hayden & Dolan (1976) using molluscs, decapod crustaceans and ascidians. Environmental conditions thus appear to have resulted in shorter geographic ranges for most other gastropod species along this coastline; *Thais emarginata* appears unusually resistant to those forces influencing range determination or speciation.

TABLE 3. Inheritance of shell color in *Thais emarginata*. Parental phenotypes and frequencies of offspring phenotypes are presented for between- and within-population crosses. Color abbreviations: BL = black, OR-BRN = orange-brown, OR = orange, GR-OR = grey-orange, GR = grey, WH = white. Source population abbreviations: B = Bamfield, British Columbia, Canada, TB = Torch Bay, Alaska, U.S.A. Tabled values are numbers of progeny. Question marks indicate uncertain scoring.

		Parent		F1 Phenotypes					
Cross	Sex	Phenotype	Source	BL	OR-BRN	OR	GR-OR	GR	WH
1	m	OR	TB	10	6		2?		
	f	BL	B	10	7		1?		
2	m	OR	TB	9	5	23?			
	f	BL	B	11	12	26?			
3	m	BL(BRN?)	B		3?		4?		
	f	OR	TB		3?		5?		
4	m	BL(BRN?)	B	3	4		6?		
	f	OR	TB	2	3		2?		
11	m	BL	TB					1?	
	f	WH	B					2?	
13	m	WH	B	10					9
	f	BL	TB	7					10
14	m	WH	B						1
	f	OR-BRN	TB			1			
15	m	WH(GR?)	TB	3	1	2			
	f	OR-BRN	TB	1	4	3			
16	m	BL	TB	6	4				
	f	OR	TB		3				
17	m	OR	B	13		14			
	f	BL	B	13		14			
18	m	BL	B	5	1?	5			
	f	OR	B	11	1?	6			

This observation is in rather sharp contrast to the patterns observed by Shuto (1974) and Hansen (1978, 1980) that species with low dispersal (lecithotrophic development) tend to have shorter geological durations and geographic ranges than those with greater dispersal (planktotrophic development). In *Thais emarginata* the lack of a pelagic larva is associated with a wide geographic range. While it is not surprising that species with greater dispersal ability should have larger geographic ranges, the claim that reduced dispersal ability precludes a wide geographic range (Shuto, 1974; Hansen, 1978, 1980) appears unsubstantiated.

Gene flow

As for many other mid- to high-latitude prosobranchs, *Thais emarginata* has direct development from benthic egg capsules (Thorson, 1950; Lyons & Spight, 1973). This species breeds in pairs, or groups of 5 or 6, and lays small clusters of egg capsules (10 to 20 per snail) in crevices or under algae (personal observation). Hatchlings leave the egg capsules after approximately 3 months development. Active dispersal thus is accomplished solely by movements of animals along the shore (but see below).

The distributional data and results of the vertical transplants revealed not only that *T. emarginata* occurs high on rocky shores (Fig. 2), but that this distribution is actively maintained (Table 1). *T. emarginata* is also normally absent from protected bays or inlets (Fig. 3), except in areas where there are strong tidal currents (personal observation). As a consequence, expanses of deep water and protected bays or inlets should act as barriers to gene flow. Since the Pacific coast from Alaska through British Columbia is

TABLE 4. Inheritance of banding in *Thais emarginata*. Parental phenotypes and frequencies of offspring phenotypes are presented for between- and within-population crosses. Source population abbreviations: B = Bamfield, British Columbia, Canada, TB = Torch Bay, Alaska, U.S.A. Tabled values are numbers of progeny.

Cross	Sex	Parent		F1 Phenotypes	
		Phenotype	Source	Banded	Unbanded
1	m	Banded	TB	12	6
	f	Banded*	B	11	7
2	m	Banded	TB	14	22
	f	Banded**	B	23	26
3	m	Unbanded	B	4	3
	f	Banded**	TB	6	2
4	m	Unbanded	B	13	
	f	Banded	TB	7	
11	m	Banded	TB		1
	f	Unbanded	B		2
13	m	Unbanded	B	10	9
	f	Banded	TB	7	10
14	m	Unbanded	B		1
	f	Banded	TB	1	
15	m	Unbanded	TB	6	
	f	Banded	TB	8	
16	m	Banded	TB	10	
	f	Banded*	TB	3	
17	m	Banded	B	15	12
	f	Unbanded	B	10	17
18	m	Unbanded	B		11
	f	Unbanded	B		18

*Unbanded when small.
**Banding very weak.

TABLE 5. Inheritance of shell sculpture in *Thais emarginata*. Sculpture was measured as the depth (mm) of the grooves between the middle spiral ribs of the body whorl at the lip of the aperture. F1 offspring were ranked visually into four approximate sculpture categories: SM = smooth (<0.14 mm), WK = weak (0.15–0.29 mm), MOD = moderate (0.30–0.44 mm), STR = strong (>0.45 mm). Tabled values are numbers of progeny.

Cross	Parental Sculpture(mm)		F1 Phenotypes			
	Male	Female	SM	WK	MOD	STR
1	0.30	0.00		13	23	
2	0.40	0.00		21	39	25
3	0.00	0.40		3	10	2
4	0.00	0.40		6	5	7
11	0.30	0.10			1	2
13	0.05	0.40		19	17	
14	0.30	0.25			1	1
15	0.35	0.40				14
16	0.40	0.50				13
17	0.05	0.15	40	14		
18	0.35	0.30	14			17

heavily dissected by fjords and estuaries, it seems likely that gene flow among these populations on the scale of hundreds of kilometers is extremely low.

It is, of course, almost impossible to document rigorously the absence of gene flow, since migration of as few as one individual per population per generation to an adjacent population can be adequate to maintain uniform gene frequencies over an extensive geographic range in the absence of selection (Lewontin, 1974). However, I believe several hypotheses about passive mechanisms of gene flow may be ruled out or minimized. First, movement by birds is unlikely to be important. While adult *Thais emarginata* may be taken from the intertidal by gulls, crows or oystercatchers to be consumed on adjacent rocks, they are usually taken above the high-tide line (Zach, 1979; personal observation) and would thus die whether they were eaten or not. Second, movement of egg capsules by dislodgment is also probably unimportant. If capsules were dislodged, they would most likely either be consumed by intertidal anemones (Sebens, 1977), drift into the strand line and die, or settle into the subtidal among drift algae where hatchlings probably would not survive.

A mode of dispersal which might occur but which would be very difficult to document is transport of hatchlings or very small juveniles on drifting algae (e.g. *Fucus*). Although drifting algae would also end up in the strand line, a juvenile could be dislodged from the alga on another shore before its final deposition. The lack of data for any of these modes precludes a definitive conclusion regarding actual levels of gene flow. The most promising test of this hypothesis of extremely low gene flow will be an analysis of the geographic distribution of rare, electrophoretically detectable alleles following the procedure of Slatkin (1981). If sufficient electrophoretic variation is present, such an analysis would provide a direct measure of the rates of gene flow in *Thais emarginata*. Preliminary electrophoretic scanning is underway.

Genetic divergence

Patterns of egg-capsule production and juvenile survivorship for between- and within-population crosses from Alaska and Vancouver Island (Table 2) suggest that in spite of reduced gene flow, there has not been sufficient genetic divergence on this geographic scale to reduce F1 viability substantially, even though physical and ecological conditions change substantially over this range (Hayden & Dolan, 1976). Crosses in progress will provide information on F2 viability. To date (July, 1982), 7 of 23 mated pairs of F1 progeny from between-population crosses have produced egg capsules and several clutches are clearly developing. In addition, crosses have been established with *T. emarginata* collected from Santa Barbara, California (U.S.A.) to try to assess the degree of divergence on a larger geographic scale. It thus appears either that gene flow is greater than expected or that selection has not been strong enough to induce genetic differentiation which would influence viability of between-population crosses. Alternatively, these data could be interpreted as consistent with the currently popular view that species are entities resistant to change except during the speciation process (Schopf, 1981; Gould, 1982).

Perhaps the most interesting pattern observed among broods was that difficulties scoring offspring color phenotypes were restricted largely to between-population crosses (e.g. 1, 2, 3, 4, and 11; Table 3). In crosses 1 and 2, many of the darker morphs that were difficult to score appeared to have a superposition of a lighter band color (orange) upon a ground shell color of black: spiral ribs were black and grooves in between appeared to be an uneven mixture of orange and black. In banded individuals, ribs most commonly are unpigmented. The four within-population crosses (15–18) exhibited more discrete color morphs. One interpretation of this pattern is that the integration of loci which regulate production of pigment and control banding is different in these distant populations, and crosses between these populations may have resulted in a breakdown of this regulation. Backcrosses and crosses in progress among F1 progeny will shed additional light on this hypothesis of disrupted regulation.

Confidence of paternity

It is well known that prosobranch gastropods have the capacity to store sperm for periods of from weeks to months (Fretter & Graham, 1962). Thus, to interpret patterns of inheritance accurately it is necessary to know either that females were virgin when established in pairs or that any stored sperm had been resorbed or become inviable. To guard against the possibility of stored sperm I used animals that were approximately half

TABLE 6. Independent assortment of banding, color and sex, and sculpture, color and sex in *Thais emarginata* crosses 17 and 18. Tabled values are numbers of progeny.

		Banding Cross 17		Sculpture Cross 18	
Color	Sex	Unbanded	Banded	Smooth	Sculptured
Orange	m	6	8	2	3
	f	11	3	3	3
Black	m	6	7	4	1
	f	6	7	4	7

adult size (mean initial shell length of parents when isolated = 13.8 ± 2.2 mm, range = 11.8–15.3 mm; mean shell length of laboratory grown adults = 25.5 ± 3.1 mm, range = 22.4–29.6 mm). The possibility that copulation occurred prior to isolation, though, cannot be ruled out entirely.

There is evidence, however, suggesting that females in these crosses produced offspring using only the sperm of the male with which they were caged. In a recent backcross where a mature female was caged with a male offspring half its size, the female produced three successive clutches at approximately one month intervals. In the first of these clutches, nearly all capsules appeared to contain developing embryos. In the second, only about 10%, and in the third none of the eggs exhibited normal development. Storage of viable sperm thus would appear limited to a period of approximately two to three months. Since none of the initial pairs produced capsules until over three and a half months after isolation, it seems likely that the progeny were derived only from the isolated parents. Mature females brought into the laboratory and fed usually produce capsules within one month or less of collection (personal observation). Crosses are currently underway between mature females and males with known color markers to determine the length of sperm storage in *Thais emarginata.*

Genetics of color, banding and sculpture

Although color, banding and sculpture polymorphisms have been described for many marine, prosobranch gastropods (Moore, 1936; Struhsaker, 1968; Berry & Crothers, 1968, 1974; Spight, 1973, 1976; Cole, 1975; Campbell, 1978; Reimchen, 1979; additional refs. in Vermeij, 1978), the genetic basis of these polymorphisms appears to have been documented for only a few species. Struhsaker (1968) found that offspring of different sculpture morphs in *Littorina picta* tended to resemble the average parental phenotype as did Newkirk & Doyle (1975) for shell shape in *Littorina saxatilis,* but phenotypes were not discrete and no genetic model was proposed. Juvenile shell color in *Urosalpinx cinerea* was explained by a tri-allelic, single locus model (Cole, 1975). In this species, however, as in *T. emarginata,* juvenile shell colors were largely transient. Thus the precise genetic basis of adult coloration and sculpture of the shell remains to be determined.

In *Thais emarginata,* F1 phenotype frequencies (Tables 3–6) suggest that there is a simple genetic basis to several aspects of variation in shell color, banding and sculpture. Mendelian ratios from crosses between black and orange (crosses 17 and 18), and black and white individuals (cross 13, Fig. 5) suggest minimally that there is a single locus with three discrete color alleles which controls adult coloration. The lack of orange offspring from crosses 1 and 16 (Table 3) indicates that black can partially or completely override the contribution of the orange allele in a heterozygote.[2] White also appears to be recessive to black and orange since only black, orange-brown and orange progeny were produced in cross 15 even though one parent was white (Table 3). The parental phenotypes of this cross, however, were not distinct and the interpretation is thus tentative.

Not all color phenotypes were clearly defined. In particular, several shades of brown and orange-brown appeared to intergrade (crosses 1–5, 15 and 16; Table 3). These could be black/orange heterozygotes or they may indicate the existence of more than one black allele or perhaps the presence of alleles

[2]See Footnote 1 (Abstract).

for brown coloration. Alternatively, more than one locus may be involved; other loci may affect hue or intensity as described for the pulmonates *Partula taeniata* (Murray & Clarke, 1976) and *Cepaea nemoralis* (Cain & Sheppard, 1957). Additional crosses will be required to clarify the number of alleles and loci controlling adult coloration in *Thais emarginata*.

Diet appears to have little if any effect on the shell color of *T. emarginata,* in contrast to results reported by Moore (1936) for another thaidid gastropod, *Nucella lapillus.* Young individuals of both *T. emarginata* (mean initial shell length = 13.7 mm) and *T. canaliculata* (16.8 mm) held in cages at two tidal heights in the field (+ 0.15 m and + 0.76 m; Friday Harbor Laboratories, Friday Harbor, Washington, U.S.A., 48°33′ N, 123°01′ W) and fed pure diets of barnacles *Balanus glandula* for approximately one month (25 and 33 days respectively) followed by a pure diet of mussels *Mytilus edulis* for approximately one month (32 and 25 days respectively) exhibited at most very slight changes in the hue of the shell even though they increased substantially in total weight both on barnacles (mean ± SE, N = 8: 141.1 ±4.07% and 99.6 ± 7.65% for *T. emarginata* and *T. canaliculata* respectively) as well as on mussels (51.2 ± 9.06% and 50.2 ± 2.93% respectively; Palmer, unpublished). A similar result was obtained with 100 individuals of juvenile *T. emarginata* (shell length range 12.7–16.9 mm) collected from Wizard Rock (Vancouver Island, British Columbia, Canada; 48°53′ N, 125°09′ W and grown nearly 360° (one full whorl) on average on either a pure diet of barnacles (50 animals on *B. glandula*) or mussels (50 on *M. edulis*) over a period of 58 days while continuously immersed in running seawater in the laboratory. In neither experiment was a dramatic loss of pigment observed in individuals with dark-colored shells when grown on barnacles, or an increase observed in shell pigmentation when grown on mussels.

In a widely cited work, Moore (1936) reported that dark-shelled *N. lapillus* produced white shell when transferred to a diet of barnacles in the laboratory and in the field, and also that the % pigmented shells in natural populations was significantly correlated with the availability of *Mytilus edulis* as food. His conclusion that brown/black or mauve shell color is a product of consuming *Mytilus edulis,* however, is open to question on several grounds (see also Berry & Crothers, 1968, 1974). First, no pigmentation was observed to appear in white-shelled animals from a *Balanus* fed community that were grown on mussels even though they were fed mussels for six months (Moore, 1936: 80). Second, I have observed many instances of abrupt shell-color changes in animals from the field as well as those transferred to and grown in the laboratory; these changes, however, were almost invariably from darker shell to either a lighter or an unpigmented shell. In the laboratory, these changes in shell color appeared to result from some kind of trauma as they were associated with an abrupt cessation and reinitiation of growth. Thus, the cessation of pigmentation observed by Moore in some transplanted snails may have been due to the trauma of transplantation rather than a change in diet. Further, these animals often regained their previous pigmentation if grown long enough, a pattern Moore concluded was due to a return to a mussel diet. The lack of quantitative data on frequency and intensity of the experimentally induced color changes makes Moore's results difficult to evaluate. Third, the observed correlations between shell color and availability of *Mytilus* on the shore need not have been produced by dietary differences; they could have resulted from differential mortality due to visual predators. Finally, among the studies with which I am familiar, all of the other reported cases of conspicuous shell-color change with diet are for archaeogastropods [*Turbo cornutus* (Ino, 1949), *Haliotis rufescens* (Leighton, 1961), *H. cracherodii* (Leighton & Boolootian, 1963), *Austrocochlea constricta* (Underwood & Creese, 1976)], a pattern consistent with Comfort's (1951) interpretation that the Meso- and Neogastropoda are fundamenally different from the Archaeogastropoda in the chemistry of their pigment systems. The direct effect of diet (as opposed to rate of growth or trauma) on shell pigmentation remains to be demonstrated conclusively in meso- and neogastropods.

Banding appears to be controlled by two alleles at a single locus. Within-population crosses yielded broods that were either uniformly banded (crosses 15 and 16), uniformly unbanded (cross 18) or contained an equal number of banded and unbanded individuals (cross 17, Table 4; Fig. 6). The lack of unbanded offspring where one parent was unbanded (cross 15) and the presence of unbanded offspring where both parents were

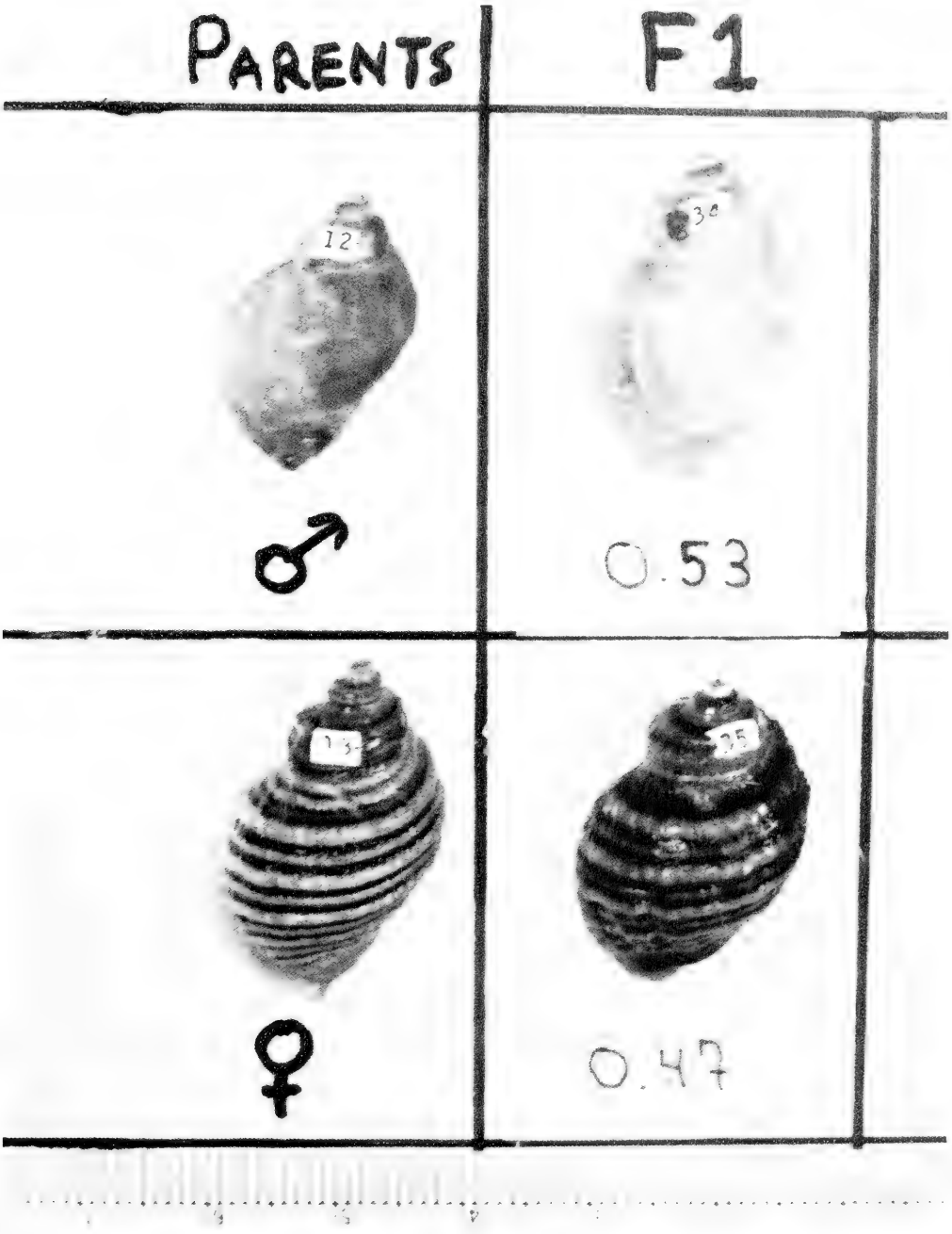

FIG. 5. Phenotypes of parents and F1 offspring from cross 13. The white male parent was from Bamfield, British Columbia, the black banded female from Torch Bay, Alaska. The total number of offspring was 36; numbers under progeny indicate proportions of each phenotype. Scale units in cm.

banded (cross 1) suggests that banding is dominant as in the pulmonate gastropods *Partula taeniata* (Murray & Clarke, 1966) and *Arianta arbustorum* (Cook & King, 1966). However, the offspring of cross 11 were all unbanded even though one parent was banded, so dominance remains to be determined decisively. Crosses 1 and 11 were both between-population crosses and, as suggested above, the integration of color and

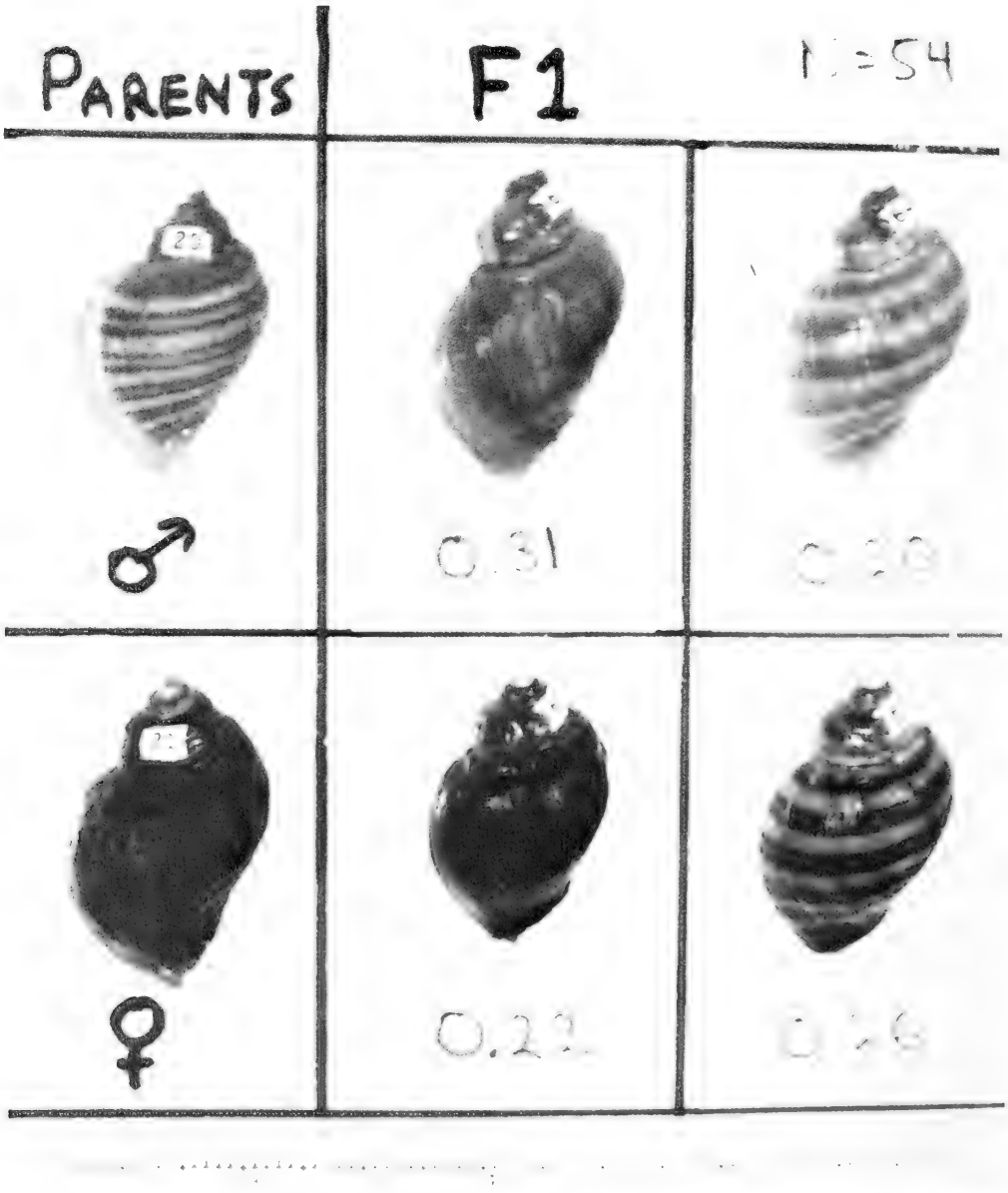

FIG. 6. Phenotypes of parents and F1 offspring from cross 17. Both parents, the orange banded male and the black unbanded female, were from Bamfield, British Columbia. F1 phenotypes, clockwise from upper left: orange unbanded, orange banded, black banded, black unbanded. The total number of offspring was 54; numbers under progeny indicate proportions of each phenotype (see also Table 6). Scale units in cm.

banding may have been disrupted in the progeny.

Perhaps the most striking outcome of these breeding experiments was the production of approximately equal proportions of two discrete sculpture morphs in the progeny of a single cross (cross 18, Table 5; Fig. 7). Expression of shell sculpture in *Thais emarginata* thus may be controlled by a single locus, or by a block of very tightly linked genes. In

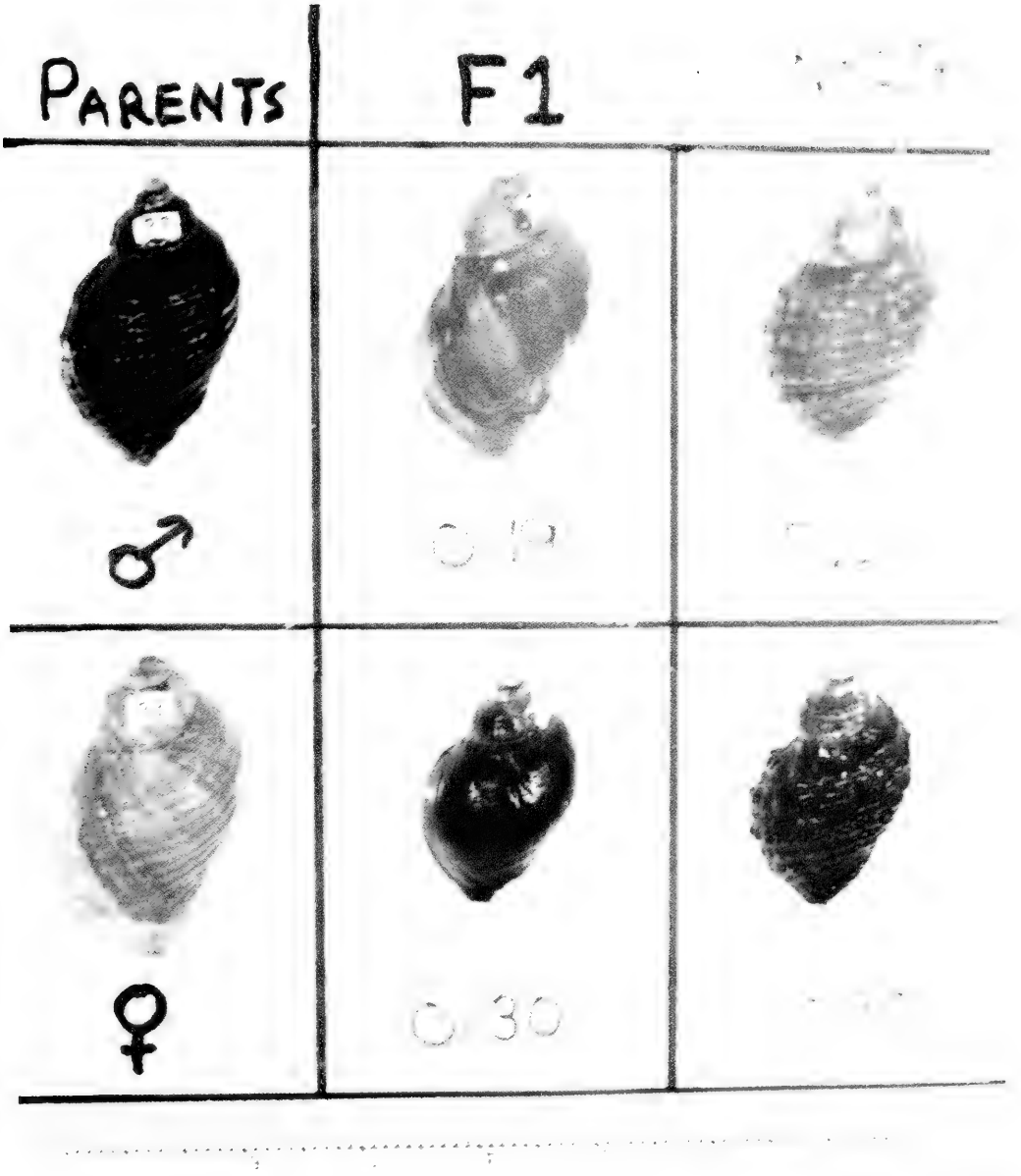

FIG. 7. Phenotypes of parents and F1 offspring from cross 18. Both parents, the black unbanded male and the orange unbanded female, were from Bamfield, British Columbia. F1 phenotypes, clockwise from upper left: orange smooth, orange sculptured, black sculptured, black smooth. The total number of offspring was 27; numbers under progeny indicate proportions of each phenotype (see also Table 6). Scale units in cm.

nearly all other crosses, an intermediate range of sculpture was produced (Table 5), suggesting that several genes actually are involved. There is strong evidence that the development of sculpture can be suppressed by environmental cues in *Thais lamellosa* (Palmer, unpublished); thus *T. emarginata* may have a similar control. If true, the discrete sculpture morphs of cross 18 may represent two alleles at a locus involved with sensing or responding to environmental stimuli rather than coding for sculpture directly.

Brood sizes were too small in most cases to assess intermediate levels of linkage; however both banding and sculpture appeared to assort independently of color (Table 6, Figs. 6 and 7). These data indicate that shell phenotype is not controlled by a supergene as suggested for several terrestrial pulmonates (Cook & Murray, 1966; Murray & Clarke 1976a,b). There also was no consistent evidence for sex linkage of color, banding or sculpture (Tables 3–6). In cross 16, black offspring were all males (Table 3) but this pattern was not observed in any other crosses. Also, in cross 18 seven of eight black, sculptured offspring were females (Table 6). Sample sizes were sufficiently small, however, that these frequencies could have resulted by chance.

In spite of difficulties associated with typing color morphs, *Thais emarginata* appears to offer tremendous potential for understanding the genetic basis of color, banding and sculpture variation in a marine, prosobranch gastropod. Further, additional crosses between adults from more widely separated populations offer the opportunity to examine how the regulation of shell phenotypes may break down when coadapted gene complexes are mixed.

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LITERATURE CITED

ABBOTT, R. T., 1974, *American seashells,* ed. 2, Van Nostrand Reinhold, New York, [vii +] 663 p., 24 pl.

ANONYMOUS, 1982, *Tide tables 1982, west coast of North and South America.* U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Washington, DC, 231 p.

AVISE, J. C., 1976, Genetic differentiation during speciation. In AYALA, F. J. ed., *Molecular Evolution.* Sinauer Associates, Sunderland, Mass., p. 106–122.

AYALA, F. J., 1975, Genetic differentiation during the speciation process. *Evolutionary Biology,* 8: 1–78.

BERRY, R. J. & CROTHERS, J. H., 1968, Stabilizing selection in the dog-whelk *Nucella lapillus. Journal of Zoology, London,* 155: 5–17.

BERRY, R. J. & CROTHERS, J. H., 1974, Visible variation in the dog-whelk *Nucella lapillus. Journal of Zoology, London,* 174: 123–148.

BUSH, G. L., 1982, What do we really know about speciation? In MILKMAN, R. ed., *Perspectives on evolution.* Sinauer Associates, Sunderland, Mass., p. 119–128.

CAIN, A. J. & SHEPPARD, P. M., 1957, Some breeding experiments with *Cepaea nemoralis* (L.). *Journal of Genetics,* 55: 195–199.

CAMPBELL, C. A., 1978, Genetic divergence between populations of *Thais lamellosa* (Gmelin). In BATTAGLIA, B. & BEARDMORE, J. A., eds., *Marine organisms: genetics, ecology and evolution.* Plenum, New York, p. 157–170.

COLE, T. J., 1975, Inheritance of shell colour of the oyster drill *Urosalpinx cinerea. Nature,* 257: 794–795.

COMFORT, A., 1951, The pigmentation of molluscan shells. *Biological Reviews,* 26: 285–301.

COOK, L. M. & KING, J. M. B., 1966, Some data on the genetics of shell character polymorphism in the snail *Arianta arbustorum. Genetics,* 53: 415–425.

COOK, L. M. & MURRAY, J., 1966, New information on the inheritance of polymorphic characters in *Cepaea hortensis. Journal of Heredity,* 57: 245–247.

ENDLER, J. A., 1977, *Geographic variation, speciation, and clines.* Princeton University, Princeton, New Jersey, 246 p.

FRETTER, V. & GRAHAM, A., 1962, *British prosobranch molluscs.* Ray Society, London, 755 p.

GOULD, S. J., 1982, Darwinism and the expansion of evolutionary theory. *Science,* 216: 380–387.

HANSEN, T. A., 1978, Larval dispersal and species longevity in Lower Tertiary gastropods. *Science,* 199: 885–887.

HANSEN, T. A., 1980, Influence of larval dispersal and geographic distribution on species longevity in neogastropods. *Paleobiology,* 6: 193–207.

HAYDEN, B. P. & DOLAN, R., 1976, Coastal marine fauna and marine climates of the Americas. *Journal of Biogeography,* 3: 71–81.

INO, T., 1949, The effect of food on growth and coloration of the topshell (*Turbo cornutus* Solander). *Journal of Marine Research,* 8: 1–5.

LEIGHTON, D. L., 1961, Observations on the effect of diet on shell coloration in the red abalone, *Haliotis rufescens* Swainson. *Veliger,* 4: 29–32.

LEIGHTON, D. L. & BOOLOOTIAN, R. A., 1963, Diet and growth in the black abalone *Haliotis cracherodii. Ecology,* 44: 227–238.

LESSIOS, H. A., 1981, Divergence in allopatry: molecular and morphological differentiation between sea urchins separated by the isthmus of Panama. *Evolution,* 35: 618–634.

LEWONTIN, R. C., 1974, *The genetic basis of evolutionary change,* Columbia University Press, New York, 346 p.

LYONS, A. & SPIGHT, T. M. 1973, Diversity of feeding mechanisms among embryos of Pacific Northwest *Thais. Veliger,* 16: 189–194.

MARCUS, N. H., 1980, Genetics of morphological variation in geographically distant populations of the sea urchin, *Arbacia punctulata* (Lamarck). *Journal of Experimental Marine Biology and Ecology,* 43: 121–130.

MAYR, E., 1963, *Animal species and evolution.* Harvard University, Cambridge, Mass., xiv + 797 p.

McLEAN, J. H., 1966, *West American Gastropoda: superfamilies Patellacea, Pleurotomariacea, and Fissurellacea.* Ph.D. dissertation, Stanford University, Stanford, Calif., 255 p.

MOORE, H. B., 1936, The biology of *Purpura lapillus.* I. Shell variation in relation to environment. *Journal of the Marine Biological Association of the United Kingdom,* 21: 61–89.

MURRAY, J. & CLARKE, B., 1966, The inheritance of polymorphic shell characters in *Partula* (Gastropoda). *Genetics,* 54: 1261–1277.

MURRAY, J. & CLARKE, B., 1976a, Supergenes in polymorphic land snails I. *Partula taeniata. Heredity,* 37: 253–269.

MURRAY, J. & CLARKE, B., 1976b, Supergenes in polymorphic land snails II. *Partula suturalis. Heredity,* 37: 271–282.

NEWKIRK, G. F. & DOYLE, R. W., 1975, Genetic analysis of shell-shape variation in *Littorina saxatilis* on an environmental cline. *Marine Biology,* 30: 227–237.

PALMER, A. R., 1980, *A comparative and experimental study of feeding and growth in thaidid gastropods.* Ph.D. dissertation, University of Washington, Seattle, Washington, 320 p.

REIMCHEN, T. E., 1979, Substratum heterogeneity, crypsis, and colour polymorphism in an intertidal snail (*Littorina mariae*). *Canadian Journal of Zoology,* 57: 1070–1085.

SCHOPF, T. J. M., 1981, Punctuated equilibrium and evolutionary stasis. *Paleobiology,* 7: 156–166.

SEBENS, K. S., 1977, Habitat suitability, reproductive ecology, and plasticity of body size in two sea anemones, *Anthopleura elegantissima* and *A. xanthogrammica.* Ph.D. dissertation, University of Washington, Seattle, 257 p.

SHUTO, T., 1974, Larval ecology of prosobranch gastropods and its bearing on biogeography and paleontology. *Lethaia,* 7: 239–256.

SLATKIN, M., 1981, Estimating levels of gene flow in natural populations. *Genetics,* 99: 323–335.

SPIGHT, T. M., 1973, Ontogeny, environment, and shape of a marine snail *Thais lamellosa* Gmelin. *Journal of Experimental Marine Biology and Ecology,* 13: 215–228.

SPIGHT, T. M., 1976, Colors and patterns of an intertidal snail, *Thais lamellosa. Researches on Population Ecology,* 17: 176–190.

STRUHSAKER, J. W., 1968, Selection mechanisms associated with intraspecific shell variation in *Littorina picta* (Prosobranchia: Mesogastropoda). *Evolution,* 22: 459–480.

THORSON, G., 1950, Reproduction and larval ecology of marine bottom invertebrates. *Biological Reviews,* 25: 1–45.

UNDERWOOD, A. J. & CREESE, R. G., 1976, Observation on the biology of the trochid gastropod *Austrocochlea constricta* (Lamarck) (Prosobranchia). II. The effects of available food on shell-banding pattern. *Journal of Experimental Marine Biology and Ecology,* 32: 229–240.

VALENTINE, J. W., 1966, Numerical analysis of marine molluscan ranges on the extratropical northeastern Pacific shelf. *Limnology and Oceanography,* 11: 198–211.

VERMEIJ, G. J., 1978, *Biogeography and adaptation; patterns of marine life.* Harvard University, Cambridge, Mass., xi + 332 p.

ZACH, R., 1979, Shell dropping: decision-making and optimal foraging in northwestern crows. *Behavior,* 68: 106–117.

MALACOLOGIA, 1984, 25(2): 493–502

INFLUENCE OF SNAIL AGE ON GENETIC VARIATIONS IN SUSCEPTIBILITY OF *BIOMPHALARIA GLABRATA* FOR INFECTION WITH *SCHISTOSOMA MANSONI*

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ABSTRACT

Four patterns of susceptibility in *B. glabrata* for infection with *S. mansoni* have been demonstrated: I, nonsusceptible at any age; II, juvenile susceptible/adult nonsusceptible; III, susceptible at any age; IV, juvenile susceptible/adult variable. Crosses between II and III showed that adult susceptibility for *S. mansoni* PR-1 was determined at a single gene locus with nonsusceptibility dominant. Crosses between III and IV suggested that the latter carried the recessive alleles for susceptibility, but another factor modified expression so that some snails became nonsusceptible. Most of the type IV snails nonsusceptible as young adults reverted to susceptibility in old age. Some snail stocks susceptible to *S. mansoni* PR-1 at any age are juvenile susceptible/adult nonsusceptible to *S. mansoni* PR-2. Crosses suggest a third allele and additional genetic factors are involved. Crosses also suggest that some snails nonsusceptible at any age carry unexpressed the alleles for adult susceptibility. Six Puerto Rican *B. glabrata* stocks have been exposed to 13 genetically different Puerto Rican *S. mansoni* strains, involving 78 snail stock/parasite strain combinations. Of these, 51 combinations showed adult nonsusceptibility or variability.

INTRODUCTION

Genetic variations in susceptibility of *Biomphalaria glabrata* for infection with *Schistosoma mansoni* may occur between snail populations in different geographic areas, between snail populations in the same area, between individuals in the same population, and at different ages in the same individual. Variations in susceptibility with age may be common enough to be of considerable importance in transmission or control of schistosomiasis.

Newton (1953) demonstrated that snail stocks considered to be nonsusceptible on the basis of testing adult snails may be juvenile susceptible. Our early studies (Richards, 1973, 1975) with various stocks of *B. glabrata* and one strain of *S. mansoni* (NIH-Sm-PR-1) demonstrated four susceptibility patterns: type I, nonsusceptible at any age, II, juvenile susceptible/adult nonsusceptible, III, susceptible at any age, and IV, juvenile susceptible/adult variable (Fig. 1). Crosses between II and III indicated that adult susceptibility was determined at a single gene locus, with nonsusceptibility dominant. Crosses between III and IV suggested that the latter carried the recessive alleles for adult susceptibility, but another factor modified expression of susceptibility such that some snails became nonsusceptible at maturity.

When snails of the above stocks were exposed to other strains of *S. mansoni,* susceptibility patterns in some cases differed from those with PR-1 (Richards, 1975, 1976a, 1976b). Some of these differences involved variations in age of the snails.

Several factors complicate studies on the influence of age on snail susceptibility. Genetic factors determining juvenile nonsusceptibility in *B. glabrata* operate throughout the life of the snail, masking the presence of genetic factors for adult susceptibility (Fig. 1). Juvenile susceptibility is determined by a complex of several genetic factors (Richards & Merritt, 1972), the interactions of which are not clearly understood. The occurrence of genetic variability in adult susceptibility has been observed in *B. glabrata* from Puerto Rico and the Dominican Republic, and may be relatively common (Richards, 1977). Mating incompatibility between some stocks of *B. glabrata* presents problems in attempts to

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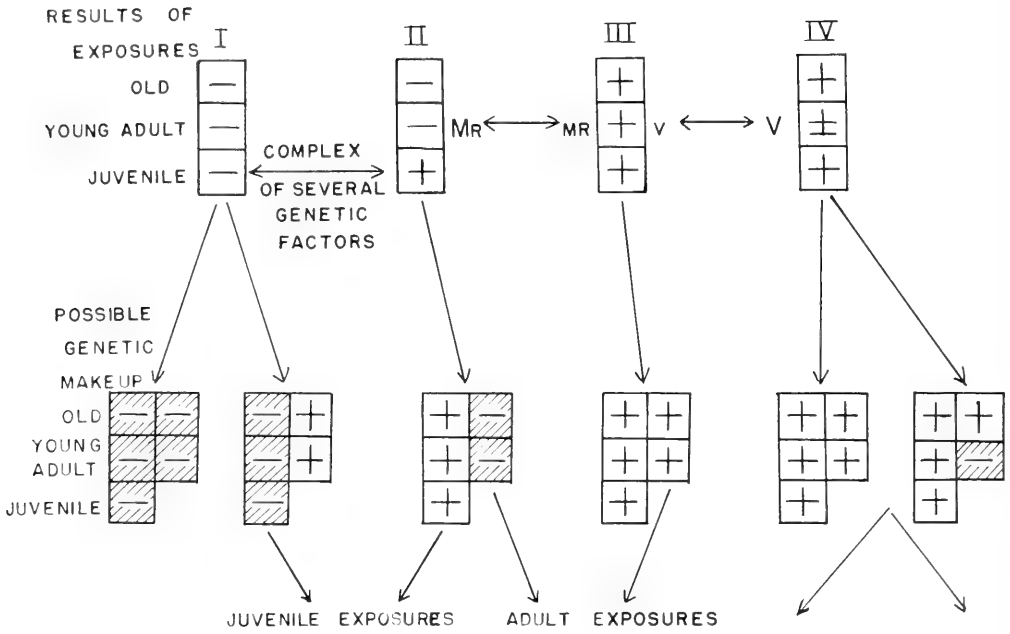

FIG. 1. Susceptibility types of *B. glabrata* for *S. mansoni* PR-1.

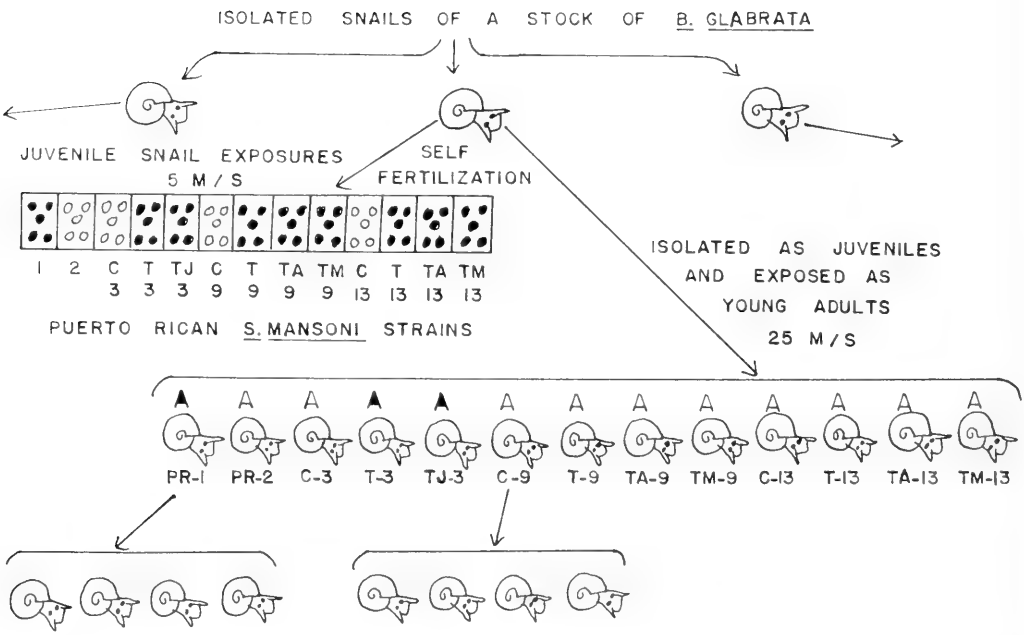

FIG. 2. Methods of testing the susceptibility patterns of a *B. glabrata* stock for a variety of *S. mansoni* strains.

determine methods of inheritance of differences in adult susceptibility.

MATERIALS AND METHODS

Snail stocks included *B. glabrata* from Puerto Rico, Brasil, St. Lucia, the Dominican Republic, and hybrids from crosses. Snails were reared singly, reproducing by self-fertilization (Fig. 2). Groups of juvenile snails from the same parent by selfing were exposed individually to different *S. mansoni* strains (5 miracidia per snail) to establish a pattern of juvenile susceptibility for the snail stock. Thirteen genetically different Puerto Rican *S. mansoni* strains were used. Snails from the same parent by selfing, isolated as juveniles, were reared to maturity as determined by onset of egglaying (Richards & Merritt, 1975). Each snail was then exposed to a different *S. mansoni* strain (25 miracidia per snail) for comparison of adult susceptibility. Juvenile offspring of exposed snails were isolated to further test for variations in adult susceptibility patterns. When snail stocks showed indications of adult variability in susceptibility, exposed young adults showing no evidence of infection were either observed over a prolonged time period for delayed parasite development or repeatedly exposed.

When juvenile and adult susceptibility patterns with respect to a series of *S. mansoni* strains were established, *B. glabrata* stocks showing susceptibility differences were crossed to determine methods of inheritance of the genetic factors involved. Pigmentation served as the main genetic marker in such crosses (Fig. 3). *B. glabrata* has, on different chromosomes, at least two genes determining pigmentation patterns, each with three alleles (Richards, manuscript in preparation). Common wildtype pigmentation (C) with black pigment in body, eyes, mantle collar, and mantle is dominant over blackeye pigmentation (c^b) with black pigment in eyes and mantle, which is dominant over albinism (c) without black pigment. On another chromosome the common allele (S) determining spotted mantle pigmentation is codominant with an allele (S^d) determining coalesced mantle pigment, both being dominant over an allele (s) for absence of mantle pigment. These alleles affect both wildtype and blackeye snails.

In a crossing experiment two snails of stocks showing different susceptibility patterns are reared in isolation (Fig. 4). Juvenile snails by precross selfing from each parent are exposed to the pertinent *S. mansoni* strains, and juveniles are isolated for adult exposures. The two parent snails are then mated for a week and reisolated. Juvenile

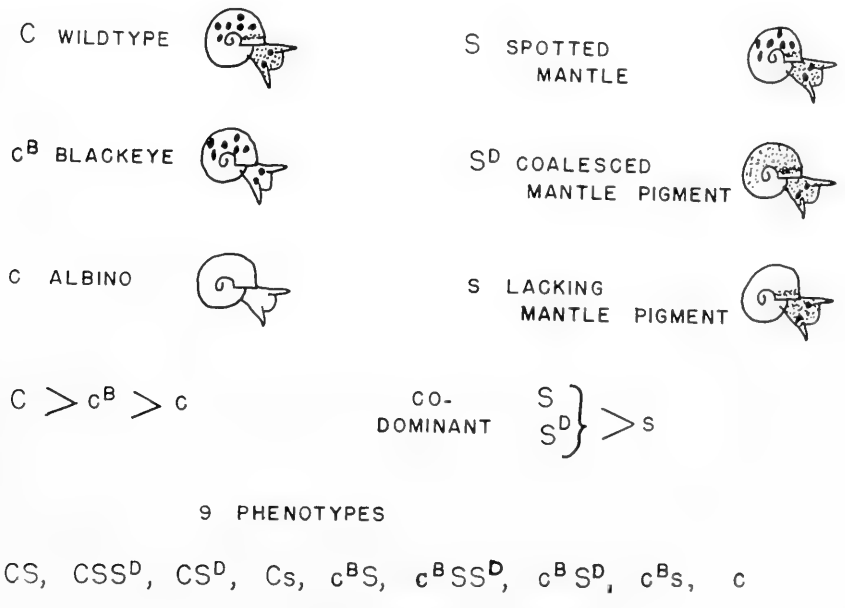

FIG. 3. Pigment markers in *B. glabrata*.

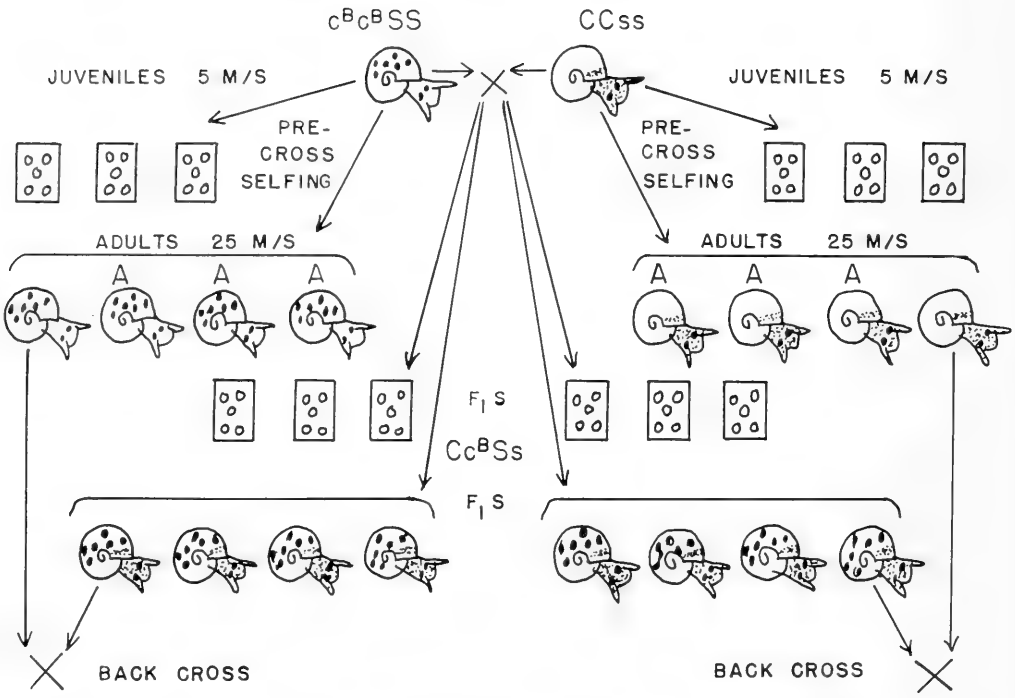

FIG. 4. Method of carrying out a snail crossing experiment to determine juvenile and adult susceptibility for various *S. mansoni* strains.

hybrid F_1s are exposed and juveniles isolated for adult exposures. Juvenile offspring of F_1s by selfing are exposed and juveniles isolated for adult exposures. Susceptible F_1s with early infections as determined by developing primary sporocysts, and nonsusceptible F_1s are backcrossed to offspring by precross selfing from each parent. The original parents, and F_1s used in crosses, are also mated to a series of snails of different susceptibility types to further characterize the inheritance of factors involved (Fig. 5). Since the success of such testing and mating procedures depend on the fecundity and mating compatibilities of the snails involved, the overall results may require the combination of several crosses.

RESULTS

Variability in adult susceptibility

In stocks showing variable adult susceptibility, selection and selfing through six generations failed to produce lines showing either consistent adult susceptibility or nonsusceptibility (Richards, 1973, 1977). A cross between albino 243432...7 (type II, juvenile susceptible/adult nonsusceptible to PR-1 *S. mansoni*) and blackeye 1-13-131...10 (type III, susceptible to PR-1 at any age) resulted in further information on variability in adult susceptibility (Fig. 6). This cross also provided several snail lines still used in studies on susceptibility and two mutations serving as genetic markers. This and other crosses have demonstrated that stock 243432 carries unexpressed the alleles for adult variability. Stock 1-13-131 does not show adult variability. The hybrid F_1s from this cross, and the first generation from the F_1s by selfing, were not exposed to *S. mansoni*. In succeeding generations, when exposures were carried out, snail lines showing the parental susceptibility types (II and III) were observed. Some lines also showed adult variability (IV). When some of the snail lines followed were tested for susceptibility for both PR-1 and NIH-Sm-PR-2, several different susceptibility patterns were demonstrated: albino stocks juvenile susceptible/adult variable to PR-1 but nonsusceptible to PR-2 (93375 and 933512); a blackeye stock juvenile susceptible/adult variable to both *S. mansoni* strains (13152-

142); a blackeye stock susceptible to PR-1 but nonsusceptible to PR-2 at any age (13141); and a blackeye stock susceptible to both strains (13142). A mutation appeared in some of the offspring of 13142-10-1 determining coalesced mantle pigment (Fig. 3). A mutation appeared in a line (831) derived from a cross between 13141 and 13142-10-1-6 resulting in a biphallic condition (see Fig. 10).

Several studies had indicated that some *B. glabrata* susceptible as juveniles to a particular *S. mansoni* strain became nonsusceptible at the onset of egglaying, but reverted to susceptibility in old age when reproductive activity had apparently decreased or terminated. In studies by Richards (1973, 1977), snails testing nonsusceptible as young adults were reexposed repeatedly. When parasites

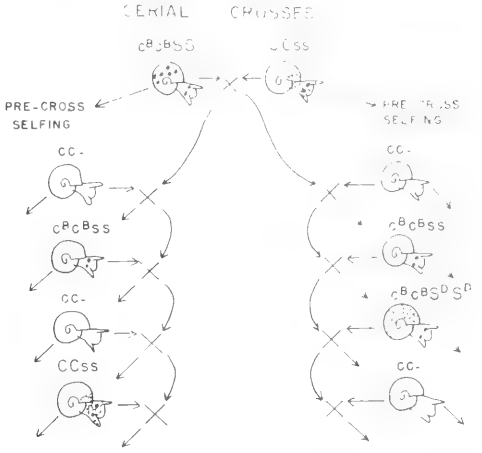

FIG. 5. Method of performing serial crosses for comparison of *S. mansoni* susceptibility patterns.

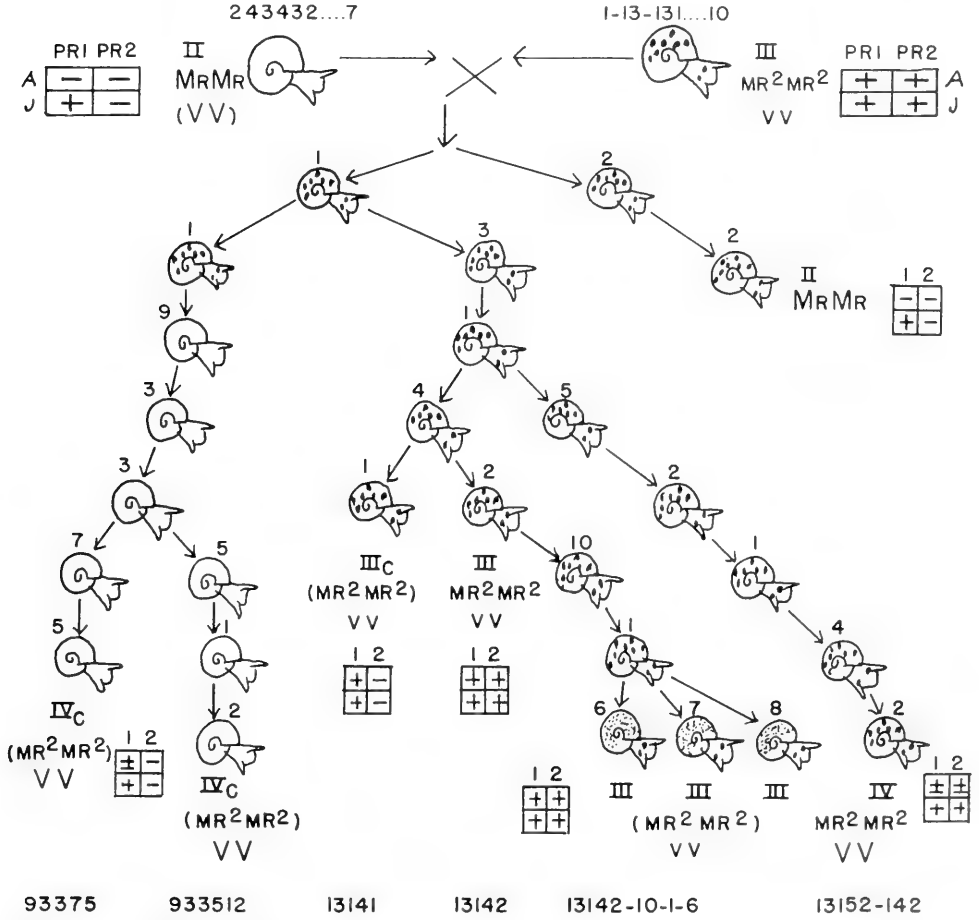

FIG. 6. Diagram of a cross between two snails resulting in lines showing five different susceptibility patterns. and inheritance of a genetic factor determining variability in adult susceptibility.

failed to develop, it was assumed they had been destroyed, but histologic studies were not done. Stock 13152-142 (Fig. 6) demonstrated juvenile susceptibility/adult variability to both PR-1 and PR-2 *S. mansoni*. In studies on this snail stock some snails apparently not infected when exposed as young adults were reexposed repeatedly while others were held and observed without reexposures. In some of the latter undeveloped but apparently viable miracidia (or young sporocysts) were seen in the tentacles. In this blackeye stock the tentacles lack black pigment and are relatively transparent. In several such snails examined periodically and not reexposed, primary sporocysts were observed to develop several months after exposure. This suggested that in some snails demonstrating a change from juvenile susceptible to nonsusceptible at the onset of egglaying, the snails were not actively resistant but temporarily unsuitable for parasite development. The parasites remained dormant for periods up to six months, then resumed development, culminating in shedding of cercariae. Results (Richards, 1973, 1977 and manuscripts in preparation) suggest that adult variability is determined by a single gene, with temporary modification of adult susceptibility during the egglaying period of a snail's life dominant over normal adult susceptibility.

Masked adult susceptibility

B. glabrata blackeye stock 10-R2 has so far tested resistant at any age to all *S. mansoni* strains to which we have exposed it. When adult snails of this stock were exposed to echinostomes and then to *S. mansoni* (Lie *et al.*, 1977a, 1977b) many proved susceptible to *S. mansoni* infection. This suggested the 10-R2 snails may carry unexpressed the recessive alleles for adult susceptibility to *S. mansoni* PR-1. A cross was carried out between 10-R2 and Albino stock 243432 (Fig. 7). Stock 243432 is juvenile susceptible/adult nonsusceptible to *S. mansoni* PR-1. Descendents of six hybrid F_1s were followed through several generations of selfing, testing both juvenile and adult snails for PR-1 susceptibility. Several lines were obtained which showed essentially 100% juvenile and adult susceptibility. The results suggest the 10-R2 stock probably carries unexpressed the recessive alleles for adult susceptibility to *S. mansoni* PR-1.

Blackeye *B. glabrata* stock 13141 is susceptible at any age to PR-1 but nonsusceptible to *S. mansoni* PR-2. This stock was mated with albino stock 6411-R44, juvenile susceptible/adult nonsusceptible to PR-2 (Fig. 8). Hybrid offspring were followed by selfing through several generations. Several lines were derived which tested 100% susceptible at any age to PR-2. These results suggest that *B. glabrata* stock 13141, while nonsusceptible to PR-2, probably carries unexpressed the alleles for adult susceptibility for PR-2.

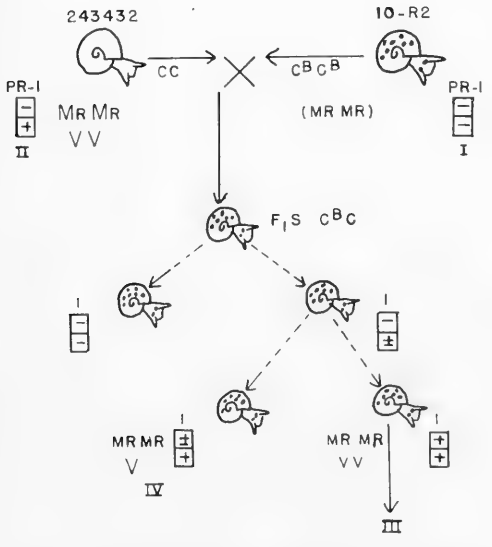

FIG. 7. Diagram of a cross demonstrating masked adult susceptibility for *S. mansoni* PR-1 in snail stock 10-R2.

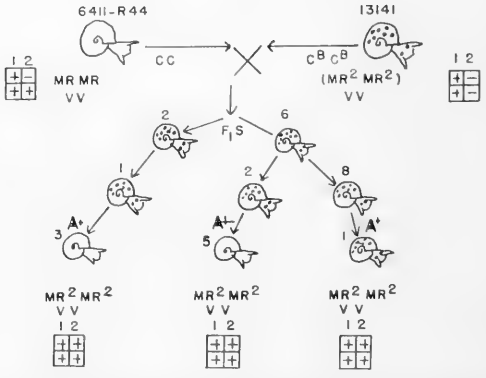

FIG. 8. Diagram of a cross demonstrating masked adult susceptibility for *S. mansoni* PR-2 in snail stock 13141.

Genetic factors for adult snail susceptibility to PR-1 and PR-2

These two Puerto Rican strains of *S. mansoni,* differing greatly in infectivity for snails, have been used in many comparative tests. PR-1 was isolated from *B. glabrata* snails collected by Dr. Elmer Berry in Arecibo in 1950. PR-2 was isolated from a stool specimen from an acute human case from Luquillo in 1975 (Fletcher *et al.,* 1981).

Previous crosses (Richards, 1973) indicated that in juvenile snails susceptible to PR-1, adult susceptibility is recessive to nonsusceptibility. *B. glabrata* stock RSF is susceptible at any age to PR-1, juvenile susceptible/adult nonsusceptible to PR-2. This suggested that either another gene was involved, or a third allele (mr^2) determined PR-2 susceptibility. When stock RSF (wild-type lacking mantle pigment) was crossed with albino 6411-R42, susceptible at any age to PR-2 and carrying alleles for mantle spotting, all the hybrid F_1s tested adult nonsusceptible to PR-2 (Fig. 9). In succeeding generations by selfing, however, several lines were derived that are adult susceptible to PR-2, as well as lines with the RSF parent susceptibility pattern.

A cross was made between stock 13141 (blackeye with spotted mantle) and stock 13142-10-1-6 (blackeye with coalesced mantle pigment) (Fig. 10). Lines from several hybrid F_1s were followed through several generations of selfing. These lines tested the same at any age; some PR-2 susceptible, some nonsusceptible. A mutation occurred in one PR-2 susceptible line, 831 with coalesced mantle pigment, resulting in a variable frequency of biphallic snails. In the course of studying this mutation, snail 831 was mated with an albino 243432 snail. When descendents of this cross were tested for *S. mansoni* susceptibility (Fig. 10) one spotted mantle blackeye line (albino 243432 carries the allele for mantle spotting) tested juvenile susceptible/adult nonsusceptible to both PR-1 and PR-2. This line (1-19-56) also showed a consistently high frequency of biphallic snails. Two snails (1-19-561 and 1-19-562) were mated several times in series to a total of four different stocks susceptible at any age to both PR-1 and PR-2. It was anticipated that all F_1s would be adult nonsusceptible to both PR-1 and PR-2. Some adult susceptible F_1s resulted from all but one of the crosses, however. F_1s from all four types of crosses were

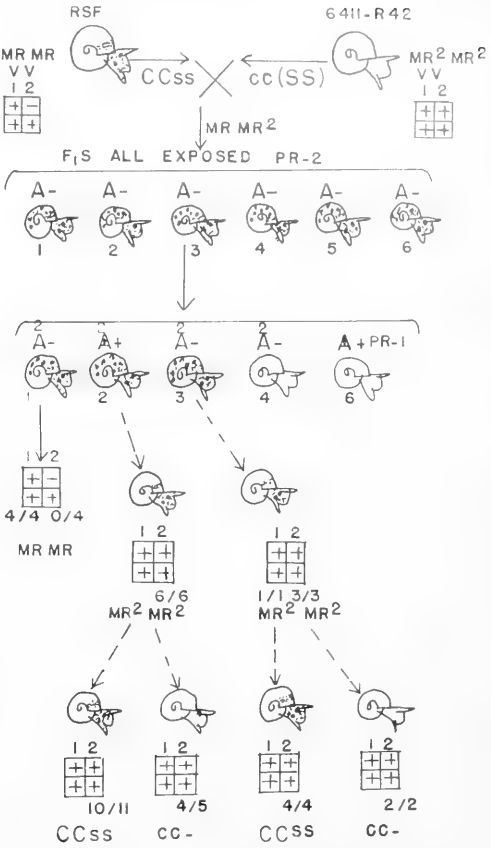

FIG. 9. Diagram of a cross suggesting that difference in adult susceptibility of *B. glabrata* for *S. mansoni* strains PR-1 and PR-2 is determined by "mr," one of three alleles (Mr,mr,mr^2).

mated with stocks adult susceptible to PR-1 but nonsusceptible to PR-2.

Fig. 11 summarizes preliminary results of two extreme examples of the crosses. When blackeye 562 was mated with albino 62525 (adult susceptible to both PR-1 and PR-2), the F_1s tested adult nonsusceptible to both PR-1 and PR-2 as expected (E). Offspring of these F_1s by selfing tested (R) 25% adult susceptible to PR-1 and 29% to PR-2, close to the expected 25%. When F_1s were mated with snails susceptible to PR-1 at any age but juvenile susceptible/adult nonsusceptible to PR-2, the resulting offspring tested 53% adult susceptible to PR-1 and 0% to PR-2.

When both blackeye 561 and 562 were mated with snails of albino stock 3234 (adult susceptible to both PR-1 and PR-2), 50% of

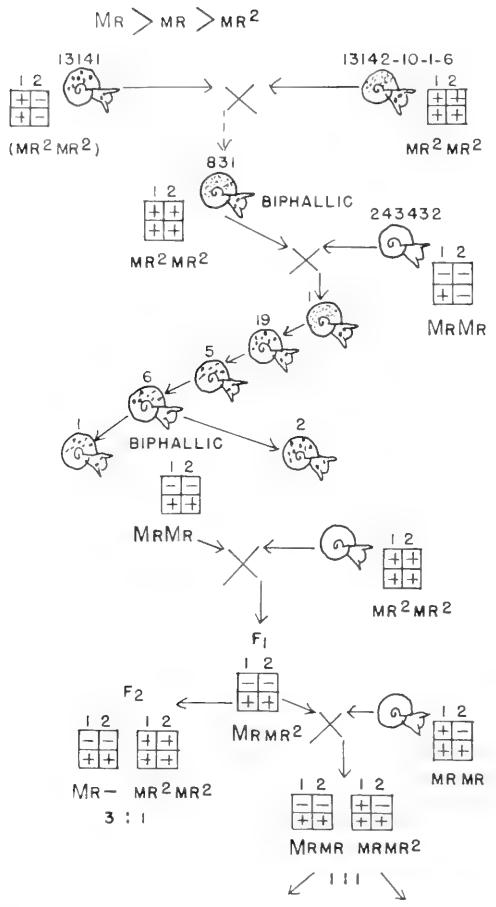

FIG. 10. Diagram of crosses involving snail line 1-19-56 to further test the concept of 3 alleles: "Mr" adult nonsusceptible to both PR-1 and PR-2, dominant over; "mr" adult susceptible to PR-1 but nonsusceptible to PR-2, dominant over; "mr²" adult susceptible to both strains.

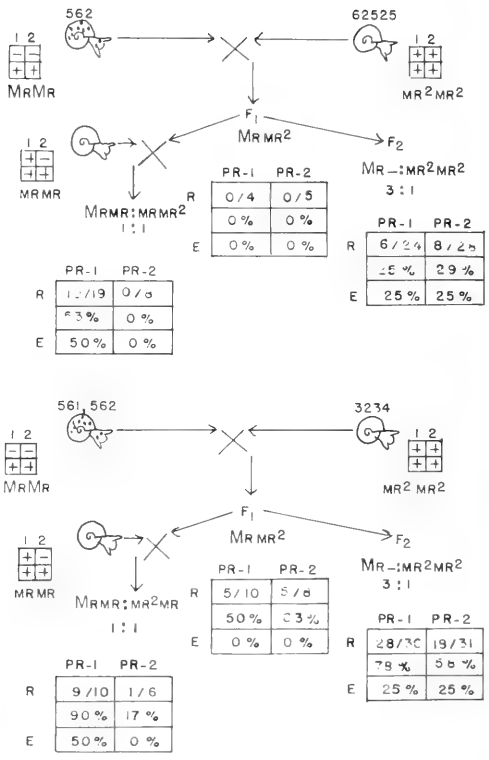

FIG. 11. Diagram of results of crosses based on Fig. 10, showing examples that fit and do not fit expected results, suggesting the involvement of additional genetic factors: results (R), expected (E).

the exposed F_1s tested adult susceptible to PR-1 and 61% to PR-2, in contrast to the expected 0% for both. Offspring of the F_1s by selfing tested 78% and 58% susceptible for PR-1 and PR-2, in contrast to the expected 25%. When several of the F_1s were mated to snails adult susceptible to PR-1 but not to PR-2, 90% of the offspring tested susceptible to PR-1 in contrast to the expected 50% and 17% to PR-2 in contrast to a 0% expectation.

These results indicated that while snail stocks 62525 and 3234 both test susceptible to PR-1 and PR-2 at any age, they differ genetically as revealed in crosses with the same snail 562. Further studies are needed to clarify the genetics of adult susceptibility of *B. glabrata* for PR-1 and PR-2. Studies are also in progress with *B. glabrata* stocks showing adult susceptibility differences for two other Puerto Rican *S. mansoni* strains, suggesting the involvement of additional genes or alleles.

Puerto Rican B. glabrata *versus Puerto Rican* S. mansoni

Six genetically different stocks of *B. glabrata* from different localities in Puerto Rico have been compared for susceptibility as both juveniles and adults for infection with each of 13 genetically different Puerto Rican *S. mansoni* strains (Table 1). In addition to differences in infectivity patterns for various *B. glabrata* stocks, allozyme differences have been demonstrated in most of these Puerto Rican *S. mansoni* strains (Fletcher *et al.*, 1981). *B. glabrata* stock PR-77 has tested susceptible at any age to all strains of *S. mansoni* so far tested. PR-78 tested juvenile susceptible/adult variable to all 13 Puerto Rican *S. man-*

TABLE 1. Patterns of susceptibility of six stocks of Puerto Rican *B. glabrata* for thirteen Puerto Rican strains of *S. mansoni*.

	Number of *S. mansoni* strains involved in each susceptibility pattern						
Adult	+	V	–	±	–	–	Total
Juvenile	+	+	+	±	±	–	combinations
Stocks of *B. glabrata*							
PR-77	13						13
PR-78		13					13
PR-79	4	7	2				13
PR-81-2		12	1				13
RSF	3	1	2	2	5		13
PR-80	4	1		3	2	3	13
Total	24	34	5	5	7	3	78

soni strains. Many young adults testing nonsusceptible became infected when later reexposed, suggesting that these snails carry the genetic factor for variability. PR-79 tested juvenile susceptible for all 13 strains, adult susceptible for 4, adult variable for 7, and adult nonsusceptible for 2 strains. PR-81-2 tested juvenile susceptible to all 13 strains and adult variable with 12 strains. The number of snails of this stock tested is small, and like PR-78 it is probable that all the snails carry the genetic factor for adult variability. Stock RSF shows a wide range of snail-parasite relations, with some juvenile susceptibility for all 13 strains. Stock PR-80 has tested nonsusceptible at any age for 3 strains.

These Puerto Rican snail stocks and parasite strains involve a total of 78 snail-parasite combinations. Of these, 51 combinations showed adult nonsusceptibility or variability. At least three of the snail stocks (PR-77, PR-78, and PR-80) are from localities known to have been endemic recently for *S. mansoni*.

DISCUSSION

Susceptibility of juvenile *B. glabrata* appears to be determined by a complex of several genetic factors (Richards & Merritt, 1972). Combinations of genetic factors resulting in susceptibility (or nonsusceptibility) may differ in various snail stocks. Our results suggest that snails nonsusceptible as juveniles remain so throughout their lives (Fig. 1). Snails susceptible as juveniles, however, may become nonsusceptible at maturity as a result of other genetic factors (Richards, 1973). In some snail-parasite combinations studied, adult susceptibility appears to be determined by a single gene, possibly with several alleles, and with adult nonsusceptibility dominant. In other snail-parasite combinations adult susceptibility appears to involve either additional genes or incomplete dominance of nonsusceptibility.

In snail-parasite combinations demonstrating adult variability, some snails remained susceptible at maturity while others became nonsusceptible (Richards, 1973, 1977). Many of these snails nonsusceptible as young adults reverted to susceptibility in old age. Snail-parasite combinations in which the snails are susceptible as juveniles and in old age, but nonsusceptible during their active egglaying period, may favor both parasite transmission and snail survival. Results with Puerto Rican snails and parasites suggest that the occurrence of juvenile susceptible/adult nonsusceptible (or variable) snails in endemic *S. mansoni* localities may not be uncommon. Field collections made in surveillance for occurrence of naturally infected snails or for laboratory testing for snail susceptibility should include both juvenile and adult snails.

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LITERATURE CITED

FLETCHER, M., LoVERDE, P. T. & RICHARDS, C. S., 1981, *Schistosoma mansoni*: electrophoretic characterization of strains selected for different levels of infectivity to snails. *Experimental Parasitology,* 52: 362–370.

LIE, K. J., HEYNEMAN, D. & RICHARDS, C. S., 1977a, Studies on resistance in snails: interference by nonirradiated *Echinostoma* larvae with natural resistance to *Schistosoma mansoni* in *Biomphalaria glabrata. Journal of Invertebrate Pathology,* 29: 118–125.

LIE, K. J., HEYNEMAN, D. & RICHARDS, C. S., 1977b, *Schistosoma mansoni*: temporary reduction of natural resistance in *Biomphalaria glabrata* induced by irradiated miracidia of *Echinostoma paraense. Experimental Parasitology,* 43: 54–62.

NEWTON, W. L., 1953, The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabratus. Experimental Parasitology,* 2: 242–257.

RICHARDS, C. S., 1973, Susceptibility of adult *Biomphalaria glabrata* to *Schistosoma mansoni* infection. *American Journal of Tropical Medicine and Hygiene,* 22: 749–756.

RICHARDS, C. S., 1975, Genetic factors in susceptibility of *Biomphalaria glabrata* for different strains of *Schistosoma mansoni. Parasitology,* 70: 231–241.

RICHARDS, C. S., 1976a, Genetic aspects of host-parasite relationships. *Symposia of the British Society for Parasitology,* 14: 45–54.

RICHARDS, C. S., 1976b, Variations in infectivity for *Biomphalaria glabrata* in strains of *Schistosoma mansoni* from the same geographic area. *Bulletin of the World Health Organization,* 54: 706–707.

RICHARDS, C. S., 1977, *Schistosoma mansoni*: susceptibility reversal with age in the snail host *Biomphalaria glabrata. Experimental Parasitology,* 42: 165–168.

RICHARDS, C. S. & MERRITT, J. W., Jr., 1972, Genetic factors in the susceptibility of juvenile *Biomphalaria glabrata* to *Schistosoma mansoni* infection. *American Journal of Tropical Medicine and Hygiene,* 21: 425–434.

RICHARDS, C. S. & MERRITT, J. W., Jr., 1975, Variation in size of *Biomphalaria glabrata* at maturity. *Veliger,* 17: 393–395.

MALACOLOGIA, 1984, 25(2): 503–511

WHAT SHALL I MEASURE ON MY SNAILS? ALLOZYME DATA AND MULTIVARIATE ANALYSIS USED TO REDUCE THE NON-GENETIC COMPONENT OF MORPHOLOGICAL VARIANCE IN *GONIOBASIS PROXIMA*

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ABSTRACT

The pleurocerid snail *Goniobasis proxima* (Say) inhabits small streams in the piedmont and mountains of the southern Appalachians. The purpose of this study was to identify the morphological variables most useful in estimating genetic divergence between isolated populations of this snail. I made 33 measurements on ten large, mature females from each of three races of *G. proxima* using standardized techniques. These variables were screened by requiring that they vary substantially among the three races. Multivariate analysis of variance showed that 12 of the 33 measurements did not meet this requirement, including 7 of the 9 foot and body measurements. These 12 variables were generally eliminated because they had very low variances both within and between populations, although one might expect measurements on such elastic structures as the gill and osphradium to vary excessively.

The remaining 21 measurements were then made on ten individuals from each of 22 additional *G. proxima* populations. Principal component analyses were performed on both the correlation and covariance matrices of the 21 measurement variables calculated over all 250 individual snails, pooling within and between population variance. The first principal component, representing size variance, was disregarded, and the 21 measurements were ranked by their contributions to the variance on the significant principal components remaining. Among the 21 variables, there was a strong inverse correlation between variance and coefficient of variation. Not surprisingly, the variables with large means and variances were most important in the non-size, significant principal components from the covariance matrix. However, the variables with small means and variances were most important in the correlation matrix analysis. Variables of any size and from any part of the anatomy were found potentially useful, but it is recommended that all measurements be taken on structures of comparable variance. Work presented elsewhere suggests that measures of overall population divergence based on morphological variance as treated in this study are correlated with interpopulation geographic distance and environmental difference.

Key words: morphometrics; genetics; electrophoresis; divergence; mollusks; *Goniobasis*.

INTRODUCTION

Since the nineteenth century, a great deal of study has been devoted to morphometrics, and a wealth of knowledge has accumulated (reviews by Blackith & Reyment, 1971; Oxnard, 1978). One principal objective of morphometric analysis has been biological classification (reviews by Jardine & Sibson, 1971; Sneath & Sokal, 1973). However, systematists most often prefer to erect classifications based on discrete, particularly binary, characters if at all possible. The analysis of metric variables (morphometrics in the strict sense) is generally applied as a "last resort" for several reasons. First, the collection and analysis of metric data can be time-consuming, often requiring a computer and considerable statistical expertise. Second, it can be difficult to identify measurements that vary significantly. Given the number of individuals to be measured, it may be found that some metric traits do not vary at all, while others may vary excessively within groups. And finally, non-genetic variance doubtless contributes substantially to most if not all metric variation.

Non-genetic variance in metric characters

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can come from many sources. Some of the most obvious sources are extrinsic, as for example, oysters growing to fit their attachment site or soft water eroding a shell apex. Some intrinsic but not additively genetic sources of morphological variation, such as sex, reproductive condition, and health may also make significant contributions. Overall size variation is a particular problem. Although size variation surely has some genetic component, a large fraction of the variation observed in wild populations is typically due to age and nutrition. Finally, a great deal of measurement error is to be expected. In mollusks, for example, the size of the soft parts may be primarily a function of contraction due to preservation method or expansion due to the placement of a dissecting pin.

My research has recently focused on populations of *Goniobasis proxima* (Say), a pleurocerid snail living in small, isolated softwater creeks of the Appalachian Mountains and piedmont from Virginia to Georgia. In a larger study, I examined the correlations between population divergence, environmental difference, and geographic distance in order to estimate the relative importance of selection and gene flow restriction in the evolution of *G. proxima* (Dillon, 1982, 1984). Because the 25 *G. proxima* populations under study did not differ qualitatively in shell, anatomy, or karyotype, I estimated population divergence using protein electrophoresis and morphometrics. The purpose of this paper is to report the results of a screening of 33 measurement variables, in which I analytically remedy some of the problems with the application of morphometrics outlined above.

METHODS

The analysis was composed of two stages. In the first stage, I screened the morphological variables by requiring that they vary appreciably among three populations believed to be genetically different. These three populations represented the three races of *G. proxima* described by Dillon & Davis (1980) based on allozyme criteria. (They share no alleles at a minimum of two enzyme loci.) Variables failing this test fall into one of three categories. Some may simply be invariant in all *G. proxima* populations, due perhaps to developmental constraints and/or strong selection. Other measures may be so variable that values are not appreciably different between any pair of populations. This could be the result of extreme measuring error, for example. A third group of variables failing the screening procedure are those that may, in fact, vary substantially among some *G. proxima* populations other than the three selected for this test. There is certainly some chance that such variables do have a large genetic component and are thus useful for estimating genetic divergence. But because they do not vary appreciably among populations believed to be genetically different, it would also seem possible that any large differences among populations subsequently examined are environmentally induced or are the product of chance alone.

Approximately 100 individuals were collected from each of three populations representing the different races of *G. proxima.* Race A was represented by snails collected from station *Yad1,* on Naked Creek in the upper Yadkin drainage, race B was represented by station *Crip* from Cripple Creek of the New River drainage, and race C was represented by station *Phlp* from Nicholas Creek, a tributary of the Dan River. Complete locality data for these sites are given in Dillon (1982). The snails were held alive in aerated tanks at 15°C and fed commercial fish food until dissection.

From each population, 10 snails were selected using a procedure designed to eliminate some intrinsic non-genetic variance. First the largest individual in the tank was chosen. (Since these snails were collected by hand, this would be nearly the maximum size for the population.) Measurements were made on the shell, the shell was carefully cracked with pliers, and the living animal removed intact. Males, obviously parasitized individuals, or those showing reduced or discolored digestive gland were discarded and the next largest individual selected. This procedure was repeated until 10 large, healthy, sexually mature females were obtained.

Snails meeting the above criteria were placed in 70% ethyl alcohol buffered at pH 7 for exactly 5 minutes at room temperature. Afterward they were transferred to a Petri dish of water for dissection. All details of the dissection, including individual orientation and pin placement, were kept uniform. A total of 33 measurements was made on each of the 30 individuals (various methods of Davis & Carney, 1973). These variables were selected to cover a broad range of anatomical characters and with an eye toward repeatabil-

ity of measurement. They are listed in Table 1 and shown diagrammatically in Fig. 1.

The six shell measurements were made using vernier calipers. The remaining 27 measurements were made using an ocular micrometer at magnifications ranging from 12× to 100×. Length measurements were the maximum dimension of the particular organ under examination, and width measurements were generally the maximum dimension perpendicular to the length. Shell width and third whorl width were the maximum distance across the whorls (see Fig. 1), even though this was not perpendicular to shell length. Pedal ganglion diameter was the maximum dimension, generally 11 o'clock to 5 o'clock when the head of the animal was oriented towards 6 o'clock. Ganglia were fixed with Bouins solution before measurement. The jaw and radula were isolated by dissolving the entire buccal mass in commercial bleach (0.5% sodium hypochlorite).

TABLE 1. Mean and standard deviation in millimeters for measurements made on three races of *Goniobasis proxima*.

Variable number	Race A		Race B		Race C	
	Mean	SD	Mean	SD	Mean	SD
Shell						
1. Shell height (3 whorls)	14.85	.96	13.37	1.10	12.06	.58
2. Body whorl height	10.96	.55	10.51	.86	9.51	.41
3. Shell width	6.41	.33	6.73	.65	6.02	.27
4. Third whorl width	3.89	.37	3.08	.29	2.67	.29
5. Aperture length	6.38	.41	6.51	.84	5.56	.39
6. Aperture width	3.35	.22	3.88	.47	3.33	.21
External head						
7. Rostrum length	1.314	.096	1.147	.157	1.365	.136
8. Rostrum width	1.955	.137	2.182	.122	2.006	.139
9. Tentacle length	1.306	.138	1.126	.167	1.091	.117
10. Width between eyes	3.003	.159	3.144	.261	2.862	.198
11. Operculum length	4.305	.256	3.890	.503	3.546	.277
12. Operculum width	2.784	.190	2.706	.438	2.412	.333
Body						
13. Body length	23.69	2.39	22.36	3.97	18.84	2.01
14. Digestive gland length	13.23	2.63	12.40	3.07	9.70	1.13
15. Egg groove length	2.221	.332	2.381	.666	1.666	.256
16. Egg groove width	.704	.184	.651	.106	.623	.074
17. Pallial oviduct length	5.375	.466	8.218	4.01	3.954	.470
18. Pallial oviduct width	1.189	.235	1.052	.330	.755	.121
19. Gill length	5.724	.398	5.650	1.03	5.153	.629
20. Osphradium length	2.096	.224	1.846	.347	1.849	.171
21. Osphradium width	.186	.056	.178	.074	2.13	.072
Central nervous system						
22. Cerebral ganglion length	.627	.057	.721	.038	.666	.067
23. Cerebral ganglion width	.390	.043	.386	.051	.406	.040
24. Pleural ganglion length	.447	.065	.502	.070	.441	.085
25. Pleural ganglion width	.269	.021	.261	.039	.231	.022
26. Pedal ganglion diameter	.361	.028	.421	.059	.412	.056
Trophic apparatus						
27. Buccal mass length	1.963	.216	2.166	.184	2.260	.125
28. Buccal mass width	1.779	.114	2.064	.135	2.018	.118
29. Radula length	3.503	.313	4.133	.181	4.098	.247
30. Radula width	.384	.014	.417	.033	.394	.015
31. Jaw length	1.172	.065	1.270	.117	1.211	.104
32. Jaw width	.619	.021	.662	.085	.615	.056
33. 2nd. marginal tooth length	.209	.007	.244	.008	.239	.006

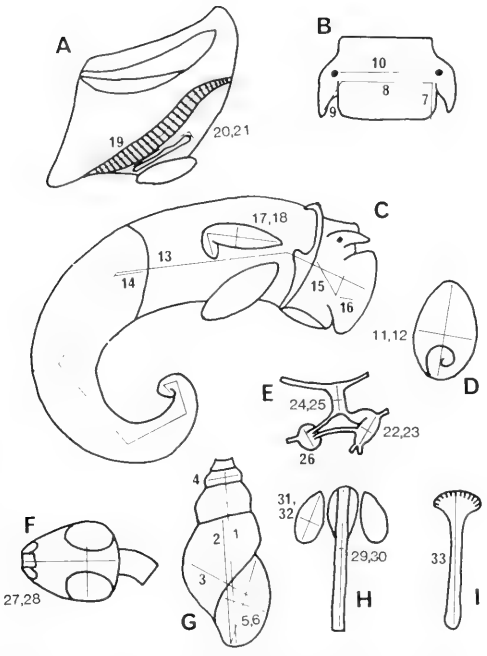

FIG. 1. Schematic diagrams showing 33 measurements made for initial screening of *G. proxima* morphological variables. See Table 1 for explanation of variable numbers. A, ventral surface of excised mantle. B, dorsal aspect of head. C, snail removed from shell. D, operculum. E, left side of central nervous sytem. F, dorsal aspect of buccal mass. G, shell. H, radular ribbon. I, second marginal tooth.

Jaws were dried flat on the slide before measurement.

A stepwise multivariate analysis of variance, BMDP7M (Jennrich & Sampson, 1981), was employed to determine if any of the metric variables varied substantially among the three races. The stepwise method was necessary because the number of variables was greater than the number of observations. At step 1, the variable selected was the one with the highest F value from an analysis of variance of the three groups. Then F values were recalculated for each of the remaining variables as in a two variable ANOVA where the variable selected in step 1 was included. At step 2, the variable with the highest F value was again selected, and F values were recalculated as in a three variable ANOVA where the variables in steps 1 and 2 were included. This process was repeated until no remaining variable had an F value corresponding to the 99% confidence level. Variables already included in the analysis of variance were removed if their F values dropped below acceptable levels during the stepping process. Only those variables with F values corresponding to the 99% confidence level at least once during the multiple stepping process were included in the second stage of the screening process.

The F value corresponding to the 99% confidence level was arbitrarily selected as a restrictive criterion. However, proper statistical inference cannot be made on these F values. Statistical inference from a multivariate analysis of variance assumes variance homogeneity. But in the 33 measurements $\times$ 3 groups = 99 separate variance estimates made for this analysis, values ranged over three orders of magnitude. Thus the true significance of these F values is unknown.

Variables passing the first stage test may still owe a great deal of their variance to simple size differences. Those variables showing the highest correlations with growth would seem most likely to be influenced by non-genetic factors such as age and nutrition. So the purpose of the second stage of this analysis was to rank the variables according to an estimate of the contribution of size.

Ten individuals from each of 22 additional populations were analyzed in the second stage (locality data in Dillon, 1982). Only a subset of the variables listed in Table 1 was examined. Otherwise the collection, dissection, and measurement techniques were identical to those used for the first three populations.

Principal component analysis has been suggested as a method of identifying some of the variance due to size, including correlated changes in shape (Blackith & Reyment, 1971; Atchley *et al.*, 1976). Principal components (PC's) can be extracted from either the covariance or correlation matrix of the measurements. If it is assumed that growth involves increase in all metric variables proportional to their coefficients of variation, analysis of the correlation matrix is appropriate. But if it is assumed that growth is best modelled as an increase in size proportional to the absolute variance of each measurement, analysis of the covariance matrix is more appropriate. Because I had no evidence that either of these assumptions was more realistic, I used both techniques to identify size-correlated variables.

Two separate principal component an-

alyses (BMDP4M, Frane & Jennrich, 1981) were performed, one on the covariance matrix of all measurements taken on the 250 individuals, the other on the correlation matrix. These were called the *covamorph* and *corrmorph* analyses, respectively. I disregarded variance on the first principal component, and tested the significance of the remaining PC's using one of two methods. For the *corrmorph* analysis, the simple rule-of-thumb method was employed that PC's with eigenvalues less than 1.0 should be disregarded. For the *covamorph* analysis, I used the method of Lawley (1956):

$$r(N-1)(\log \bar{\lambda} - \overline{\log \lambda}) = \chi^2,$$

$$\text{d.f.} = \frac{r(r+1)}{2} \quad 1$$

where N is the number of observations, r is the number of eigenvalues claimed to be equal under the null hypothesis (the smallest ones), and $\bar{\lambda}$ is the average of these last r eigenvalues. This is a test that there are no meaningful principal component directions corresponding to the last r eigenvalues. If m variables were measured, acceptance of the null hypothesis means that the first $m-r$ eigenvalues are the only ones significant.

The correlation or covariance of the original variable with a principal component is called the "loading" of that variable on that PC. Loadings adjusted by the size of the eigenvalues are measures of the contributions of particular variables to the variance represented by the PC. I used the sum of the loadings of each variable on the non-size, significant PC's as a ranking method. High-ranking variables (those with much variance uncorrelated with size) would be particularly recommended for future morphometric studies.

RESULTS

Population means and standard deviations for each of the 33 measurement variables taken on the three races are presented in Table 1. The F statistics for each variable at each of the seven steps required in the stepwise MANOVA are presented in Table 2. Twelve variables did not attain F values corresponding to the 99% confidence level at any step: shell width, aperture length, pallial oviduct length and width, gill length, operculum width, pleural ganglion length, egg groove length and width, osphradium length and width, and jaw width.

The results of the principal component analysis on the covariance matrix of the 21 remaining variables were somewhat surprising (Table 3). Principal component 1, the size component, accounted for 89.9% of the total variance, even though only the largest female snails were picked from each population in an effort to minimize variance due to size. The large eigenvalue is attributable both to high size variance and high covariances among the characters as they grow. The fact that 10 snails from each of 25 divergent populations in diverse habitats could be so similar in size relationships over a number of variables implies that measurement error was not a severe problem. Principal components 2 through 10 were found to be significant in the *covamorph* analysis, together accounting for 10.0% of the variance (Table 3). The remaining 11 components, with just 0.2% of the variance, were disregarded.

Size accounted for a much smaller portion of the variance when modelled using the correlation matrix; the first PC accounted for a relatively modest 34% of the total (Table 4). This implies that the variables with high absolute variances emphasized in the *covamorph* analysis have a higher proportion of "size" variation than do the low-variance measures. The eigenvalues of the next five PC's were also greater than 1.0, together accounting for another 39% of the variance. The last 15 principal components, accounting for 27% of the variance combined, were disregarded.

DISCUSSION

It might be expected that measurements taken on some of the hard structures, such as shell width, aperture length, operculum width, and jaw width, would not vary appreciably over three populations given a sample size of 10. However, a major result of the first stage screening was that, when properly controlled, measurements taken on even the most pliable structures can have remarkably low coefficients of variation. Examination of Tables 1 and 2 shows that, with only a few exceptions, the 12 variables were eliminated because of insufficient, not excessive, variation. It would seem advisable not to measure features of the body cavity and foot, as seven of the nine variables in that category were eliminated. But once again, even such elastic

TABLE 2. F values from stepwise multivariate analysis of variance of three *Goniobasis proxima* races based on 33 measurements. Variables above the diagonal were entered into the MANOVA. Refer to Table 1 for full names of variables.

	Step 0	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7
Degrees of freedom to enter:								
	2, 27	2, 26	2, 25	2, 24	2, 23	2, 22	2, 21	2, 20
Degrees of freedom to remove:								
	—	2, 27	2, 26	2, 25	2, 24	2, 23	2, 22	2, 21
Variables retained:								
33,MGLN	49.66	49.66	28.07	15.95	19.35	32.18	7.22	9.87
4,TWWD	26.93	13.29	13.29	18.76	17.19	5.16	4.80	4.42
31,JWLN	21.49	9.32	14.04	14.04	17.05	18.31	18.75	19.86
13,BDLN	10.95	6.98	10.53	13.21	13.21	15.52	18.36	18.22
1,SHHT	5.86	13.12	8.98	8.14	10.09	10.09	12.96	11.88
8,RSWD	8.94	6.83	9.91	8.13	7.50	10.08	10.08	13.19
7,RSLN	17.83	9.33	9.81	7.76	5.54	4.02	6.83	6.83
2,BWHT	1.40	10.28	7.75	5.06	5.63	.03	.46	.64
6,APWD	6.18	3.45	4.08	5.50	.60	2.40	1.29	3.45
9,TNLN	6.45	11.52	9.57	7.79	2.40	1.19	2.96	1.72
10,EYWD	1.99	7.36	2.05	1.07	.19	.12	.96	1.02
11,OPLN	3.43	7.11	2.11	1.13	5.11	1.18	.22	.79
14,DGLN	7.34	5.37	5.72	6.66	.13	.04	.05	.01
22,CGLN	11.58	2.50	2.62	4.01	1.06	.12	.10	.07
23,CGWD	6.85	5.37	5.03	3.81	.92	1.53	1.23	.91
25,PGWD	4.69	6.05	2.51	2.37	1.69	1.89	1.15	.98
26,PDIA	6.68	5.09	4.70	2.38	7.31	6.52	4.87	5.64
27,BMLN	6.11	.83	.67	.49	.22	.17	.90	.57
28,BMWD	14.87	.54	.58	.46	.75	3.84	1.15	1.30
29,RDLN	22.22	1.30	5.44	4.76	4.37	4.23	3.57	3.25
30,RDWD	15.83	4.63	6.32	2.24	1.24	.67	.58	.69
Variables eliminated:								
3,SHWD	1.25	1.63	1.09	1.06	.41	3.20	3.79	3.73
5,APLN	.26	2.31	.70	.75	.15	2.90	1.99	1.73
12,OPWD	.30	3.04	.98	.93	5.57	4.33	1.42	.72
15,EGLN	1.97	3.43	2.04	2.25	.01	.12	.05	.26
16,EGWD	1.95	2.92	2.67	2.42	3.75	3.65	2.45	4.19
17,POLN	3.91	.19	.50	1.32	.87	.63	.06	.06
18,POWD	.59	1.77	.61	.30	1.44	.34	.27	.51
19,GLLN	2.74	3.67	1.40	1.42	2.04	1.00	.83	.76
20,OSLN	3.09	.93	.09	.10	1.16	1.06	.25	.43
21,OSWD	.28	.26	.50	.50	.08	.11	.38	.48
24,PGLN	5.12	1.36	.96	.20	.66	.64	1.53	.42
32,JWWD	2.60	.18	1.25	2.44	2.18	3.48	1.89	1.79

and deformable organs as the osphradium and gill do not show excessive standard deviations.

The means of the 21 metric variables remaining covered a broad range of values, from 0.2 mm to 15 mm. As might be expected, the measurements with the largest means generally had the largest variances and loaded most heavily on the discarded first PC in the *covamorph* analysis. Nevertheless, these variables (e.g., shell, operculum, and body lengths) still covaried highly with the next nine PC's. The rankings computed for the 21 variables in Table 3 correspond closely to their means and absolute variances.

The results of the *corrmorph* analysis present a contrast, because the variables with the smallest means and absolute variances tended to have the largest coefficients of variation. Table 4 shows that, disregarding the first PC, the most important measurements from the correlation-based PCA were those taken on the smallest organs, such as the ganglia of the central nervous system and

TABLE 3. Results of principal component analysis on the covariance matrix of 21 *G. proxima* measurements, N = 250.

Variable name	Factor loadings										Rank
	1	2	3	4	5	6	7	8	9	10	
SHHT	.686	.766	.059	−.244	.021	.024	−.007	.036	−.013	−.062	1
BWHT	.359	.623	.027	.146	−.021	−.114	−.072	−.059	.000	.060	2
TWWD	.252	.138	.013	−.212	.033	.101	.068	−.031	.039	.100	5
APWD	.079	.270	.041	.261	−.110	.182	−.013	−.002	.013	−.010	3
DGLN	.025	−.207	.502	.026	.009	−.010	.006	−.003	.000	.000	4
BDLN	.035	−.100	−.371	.013	−.010	.006	−.010	.002	.000	.000	9
OPLN	.143	.189	−.006	.083	−.116	−.089	.157	.069	−.006	.015	6
RSLN	.026	.061	−.004	.046	.056	−.019	.006	.003	.070	−.045	12
RSWD	.043	.069	−.014	.066	.100	.021	.061	−.046	−.046	−.009	10
TNLN	.023	.036	−.011	.050	.035	−.057	.028	−.030	.112	−.020	11
EYWD	.085	.104	−.021	.107	.095	.004	.083	−.075	−.043	−.034	8
BMLN	.016	.050	−.012	.038	.037	.043	.017	.017	.054	−.011	13
BMWD	.035	.059	−.013	.057	.039	.031	.035	−.017	.002	−.002	14
CGLN	.008	.006	−.005	.013	−.009	.006	.004	−.003	.010	.001	16
CGWD	.005	.009	−.001	.004	.002	.003	.000	.001	.003	.000	21
PGWD	.003	.004	−.002	.004	−.002	.006	.002	−.004	.002	.002	19
PDIA	.006	.010	−.002	.002	−.007	.009	.005	.001	.004	.002	17
JWLN	.002	.045	.007	.053	.007	.027	−.001	.024	−.003	−.005	15
RDLN	.043	.107	−.003	.160	.178	.008	−.023	.108	−.008	.043	7
RDWD	.001	.011	−.003	.012	.002	−.001	.004	.000	.004	.001	18
MGLN	.000	.005	−.001	.010	.005	−.001	.001	.001	.002	.000	20
Eigenvalue—											
	19.240	1.195	.397	.256	.086	.073	.048	.032	.026	.023	
Cumulative variance—											
	.898	.954	.972	.984	.988	.991	.994	.995	.996	.998	

features of the head. Shell height and body whorl height, the most prominent variables in the *covamorph* analysis, ranked sixteenth and twenty-first in the *corrmorph* analysis. Conversely, the most important variable from the *corrmorph* analysis, pedal ganglion diameter and cerebral ganglion length, ranked seventeenth and sixteenth in the *covamorph* analysis.

As an overall measure of the relative contributions of size to variance in the 21 variables, one might simply sum the ranks from the *corrmorph* and *covamorph* analyses. Interestingly, the apparent inverse relationship between variance and coefficient of variation caused this summed rank to approximate 21 in most variables. The least size-influenced variable, with a summed rank of 12, was digestive gland length. Also particularly useful were body length, third whorl and aperture width, rostrum width, tentacle length, and radula length. Particularly poor, with a summed rank of 41, was cerebral ganglion width, which showed almost no variance outside the first PC. Also of reduced utility for the same reasons were buccal mass length and width, radula width, and second marginal tooth length.

In sum, it seems that useful measurements can be taken from any aspect of *G. proxima*'s morphology. Hard parts, soft parts, large items and small are all potentially valuable, with scattered exceptions. It seems important, however, that all variables measured in future studies should be of similar size and variance to the extent possible. If very large measurements and very small measurements are combined and a principal component analysis is based on their covariance matrix, variance in the small measurements may be negligible even if the first PC is discarded. If the correlation matrix is factored, the contributions of the large measurements may be negated.

If factor scores from either the *covamorph* or the *corrmorph* analysis are combined with two significant count variables (gill filaments and outer marginal tooth cusps), evidence presented elsewhere suggests that the result-

TABLE 4. Results of principal component analysis on the correlation matrix of 21 *G. proxima* measurements, N = 250.

Variable name	Factor loadings						Rank
	1	2	3	4	5	6	
SHHT	.703	.566	−.039	.016	−.098	.237	16
BWHT	.836	.228	.019	−.012	−.015	.368	21
TWWD	.384	.756	−.051	.047	−.108	−.098	10
APWD	.702	−.139	.327	.121	−.134	.298	13
DGLN	.441	.732	−.168	.003	.075	−.191	8
BDLN	.494	.718	−.170	.019	.094	−.214	7
OPLN	.669	.226	.085	.048	.168	.438	15
RSLN	.576	−.192	−.145	−.397	.243	.062	12
RSWD	.675	−.142	−.148	−.403	−.287	−.262	5
TNLN	.451	−.180	−.096	−.311	.668	.007	4
EYWD	.793	−.085	−.068	−.341	−.128	−.199	18
BMLN	.543	−.217	.200	.156	−.143	−.241	17
BMWD	.757	−.192	.047	.136	−.175	−.262	19
CGLN	.424	−.122	−.091	.670	.312	−.112	2
CGWD	.502	−.021	.167	.323	−.054	−.107	20
PGWD	.271	.014	.537	.046	.217	−.403	6
PDIA	.008	.167	.853	−.248	.078	−.055	1
JWLN	.501	−.303	.462	−.026	−.321	.185	3
RDLN	.640	−.364	−.470	.067	−.202	−.004	9
RDWD	.627	−.366	.243	.079	.281	−.010	14
MGLN	.608	−.526	−.396	.067	.066	.004	11
Eigenvalue—	7.136	2.924	1.977	1.230	1.118	1.025	
Cumulative variance—	.340	.479	.573	.632	.685	.734	

ing measures of overall morphological population divergence between the 25 *G. proxima* populations are significantly positively correlated with a number of other matrices (Dillon, 1984). First, even though the *corrmorph* and *covamorph* measures were based virtually on different sets of variables, they were found to be correlated with each other at the .001 level. Secondly, both matrices were correlated with a matrix of Rogers genetic distances between the 25 populations, calculated from allele frequencies at seven enzyme loci. Thirdly, both *corrmorph* and *covamorph* are correlated with geographic distance between the 25 populations, measured through water or over land. Finally, both matrices are correlated with environmental difference between the 25 populations, estimated using various physical, chemical, and biological variables.

These findings are necessary but not sufficient evidence that morphological data taken on *G. proxima*, analytically treated as it has been here, does in fact have some genetic component. The conclusion that can be drawn with the greatest certainty is that the measures of morphological divergence developed in this analysis are not substantially composed of measurement error.

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LITERATURE CITED

ATCHLEY, W. R., GASKINS, C. T. & ANDERSON, D., 1976, Statistical properties of ratios. I. Empirical results. *Systematic Zoology,* 25: 137–148.

BLACKITH, R. E. & REYMENT, R. A., 1971, *Multivariate morphometrics.* Academic Press, New York, 412 p.

DAVIS, G. M. & CARNEY, P., 1973, Description of *Oncomelania hupensis lindoensis,* first intermediate host of *Schistosoma japonicum* in Sulawesi (Celebes). *Proceedings of the Academy of Natural Sciences of Philadelphia,* 125: 1–34.

DILLON, R. T., Jr., 1982, *The correlates of divergence in isolated populations of the freshwater snail,* Goniobasis proxima *(Say).* Ph.D. Dissertation, University of Pennsylvania, Philadelphia, 183 p.

DILLON, R. T., Jr., 1984, Geographic distance, environmental difference, and divergence between isolated populations. *Systematic Zoology,* 33: 69–82.

DILLON, R. T., Jr. & DAVIS, G. M., 1980, The *Goniobasis* of southern Virginia and northwestern North Carolina: Genetic and shell morphometric relationships. *Malacologia,* 20: 83–98.

FRANE, J. & JENNRICH, R., 1981, Factor analysis. In: DIXON, W. J., ed., *BMDP Biomedical computer programs.* University of California Press, Berkeley, p. 656–684.

JARDINE, N. & SIBSON, R., 1971, *Mathematical taxonomy.* Wiley, London, 286 p.

JENNRICH, R. & SAMPSON, P., 1981, Stepwise discriminant analysis. In: DIXON, W. J., ed., *BMDP Biomedical computer programs.* University of California Press, Berkeley, p. 711–740.

LAWLEY, D. N., 1956, Tests of significance on the latent roots of covariance and correlation matrices. *Biometrika,* 43: 128–136.

OXNARD, C. E., 1978, One biologist's view of morphometrics. *Annual Review of Ecology and Systematics,* 9: 219–241.

SNEATH, P. H. A. & SOKAL, R. R., 1973, *Numerical taxonomy.* Freeman, San Francisco, California, 573 p.

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GENETICS OF *BIOMPHALARIA GLABRATA*: LINKAGE ANALYSIS AND CROSSING COMPATIBILITIES AMONG LABORATORY STRAINS

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ABSTRACT

The genetic basis for eleven electrophoretic phenotypes has been demonstrated through controlled crosses of the snail *Biomphalaria glabrata*. These loci plus a locus controlling body pigmentation apparently mark eight linkage groups in *B. glabrata*. Reproductive incompatibilities were observed during mating experiments with some laboratory strains of *B. glabrata*. This was especially marked in crosses involving an N.I.H. albino strain and a strain originating from the Dominican Republic. An estimate of genetic distance, based on electrophoretic markers, of 0.32 was obtained for the albino and Dominican Republic strains. This distance is comparable to those observed at the species level of *Drosophila*.

Key words: *Biomphalaria glabrata*; electrophoresis; isozymes; linkage analysis; genetic distance; mating compatibility.

INTRODUCTION

The planorbid snail *Biomphalaria glabrata* (Say, 1818) is a major intermediate host for human schistosomiasis. Laboratory studies indicate that the snail's ability to transmit schistosomes is under genetic control (Newton, 1953, 1955; Richards, 1973a, 1975b; Richards & Merritt, 1972). Transmission of *Schistosoma mansoni* (Sambon, 1907) is often limited to sympatric forms of the snail suggesting a strong degree of local coevolution (Saoud, 1965; Richards, 1973a; Michelson & DuBois, 1978). Does snail-parasite compatibility involve a few genes or many? Does the genetic variation reside with the snail or parasite or both? Answers to these questions are essential if we are to formulate strategies for control of human schistosomiasis.

C. S. Richards and coworkers have pioneered efforts to genetically dissect the basis for resistance to schistosomes in *B. glabrata* (Richards, 1973a, 1973b, 1975a, 1975b, 1976a, 1976b; Richards & Merritt, 1972). These studies indicated that juvenile resistance to specific strains of the parasite appeared to involve a number of gene loci. Adult susceptibility may be controlled by a single Mendelian locus. This work provides evidence for genetic variation in both the snail and parasite. Clearly, the requisite variability for coevolution exists in nature. Detailed studies of the genetic basis for snail susceptibility would benefit from appropriate genetic markers on a major portion if not all chromosomes.

Richards (1973b) has examined morphological markers and described five linkage groups in *B. glabrata*. Recent studies have described electrophoretic techniques for resolution of enzyme phenotypes in *Biomphalaria* (Montiero & Narang, 1976; Narang & Narang, 1974, 1976a, 1976b, 1976c; Hendricksen & Jelnes, 1980; Jelnes, 1982). We described electrophoretic techniques for resolving the phenotypes of 28 presumptive gene loci encoding enzymes and demonstrated the Mendelian basis for eight of these loci (Mulvey & Vrijenhoek, 1981a). In the present study, we examine the inheritance and linkage relationships of eleven electrophoretic loci and a pigmentation trait in *B. glabrata*. During the course of the crossing experiments, it became clear that not all strains of *B. glabrata* are compatible in terms of mating potential or F_1 and F_2 survivorship. These interstrain compatibilities are discussed in reference to estimates of overall genetic similarity among strains.

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MATERIALS AND METHODS

Electrophoresis

Snails were removed from the shell and dissected into two samples composed of 1) the head and foot and 2) the hepatopancreas and ovotestis. Tissues were crudely homogenized in 0.2–0.5 ml of a grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NADP, pH 7.0). The homogenate was centrifuged at 5000 × g for 5 min at 4°C and the supernatant was loaded into preformed slots in 12.5% (w/v) vertical starch gels. A detailed description of the electrophoretic techniques and specific enzyme stains is outlined in Mulvey & Vrijenhoek (1981a).

The genetic designations for isozymes follow the conventions established in that paper. Italics indicate genotypic designations which are common abbreviations for the enzymes under study. In multi-locus systems, loci are numbered in order of decreasing anodal mobility of the isozyme products. Isozyme mobilities were calculated relative to a bromphenol blue marker dye. In our earlier paper, the common allozyme (allelic isozyme) of the M strain of *B. glabrata* was designated *100* and other allozymes as percentages relative to the M allozyme. For the sake of simplicity in this study, we only refer to the alleles as F or S corresponding to their fast or slow migrating allozymes in the F_1 hybrids. A listing of most of these alleles is provided in Mulvey & Vrijenhoek (1981a).

Specimens

The laboratory strains of *Biomphalaria* used are shown in Table 1 along with their source and geographic origin. All stocks have been maintained in the laboratory for many generations although their histories and effective numbers are not well known. Stock snails were maintained in 4 liter aquaria containing aerated tap water with 0.5 ml/l salt solution (50 g $CaCO_3$, 5 g $MgCl_2$, 5 g NaCl, 1 g KCl per 3 liters water) and were fed romaine lettuce.

Because of the diverse geographical origins of the strains used in the crossing experiments, it was important to estimate the degree of genetic divergence between strains. Nei's (1972) index of genetic distance was calculated for all pairs of strains of *B. glabrata.* For the sake of comparison, strains of two other New-World species, *B. straminea* (Dunker, 1848) and *B. tenagophila* (Orbigny, 1835) were included in the analysis. Unfortunately, estimates of genetic distance based on laboratory strains might not precisely reflect the real distances among the source populations since these strains represent only a small sampling of the natural allelic variance. Nevertheless, they are likely to be fairly accurate given the large number of

TABLE 1. Species and strains of *Biomphalaria* used for genetic studies. Source and geographic origin are indicated.

Strain	Abbreviation	Source*	Geographic Origin
B. glabrata			
M	M	Richards	Brazil × Puerto Rico
Blackeye	I-10-R2	Cheng	M × Puerto Rico
RSF-PR1	RSF-PR1	Richards	Puerto Rico
Wild type, Puerto Rico	MPR	Richards	Puerto Rico
Antler tentacle	1-13-131	Richards	M × L-311
Pearl forming	963	Richards	Derived from M strain
Wild type, Brazil	SCP	Lewontin	Salvatore City, Brazil
Wild type, Brazil	PF	Lewontin	Parque Forestal, Brazil
Belo Horizonte, Brazil	Z	Richards	Brazil
Wild type, Brazil	Bz-C	Cheng	Brazil
L-311	L-311	Richards	St. Lucia
Dominican Republic	DR	Richards	Dominican Republic
B. straminea	I-R.59222	Richards	
B. tenagophila	BtBP	Richards	

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gene loci used in the analysis (Nei, 1978). The set of 28 enzymatic gene loci described by Mulvey & Vrijenhoek (1981a) were employed in this study. When two or more alleles were segregating at a particular locus within a strain, each allele was assigned a frequency of 1/a, where a is the number of alleles.

Of those 28 enzyme loci, 11 revealed interstrain differences that could be examined in crossing experiments. These eleven loci were: *Cat* (catalase EC 1.11.1.6); *Est-1, Est-2,* and *Est-4* (esterase EC 3.1.1.1); *Pgi* (phosphoglucose isomerase EC 5.3.1.9); *Pgd* (6-phosphogluconate dehydrogenase EC 1.1.1.44); *Pep-2* (peptidase EC 3.4.11); *Pgm-1* (phosphoglucomutase EC 2.7.5.1); *Me-2* (malic enzyme EC 1.1.1.40); *Ald* (aldolase EC 4.1.2.3); and *G6pd-1* (glucose-6-phosphate dehydrogenase EC 1.1.1.49). We were also able to score the *Pig* locus, the locus affecting albino body pigment (Richards, 1973b).

Genetic Analysis

Individual snails of each strain were isolated as juveniles from mass cultures and raised in isolation until sexual maturity. The fertility of the isolates was assured by allowing individuals to produce progeny by self-fertilization prior to crossbreeding. Mated pairs were placed in 1 liter glass jars for 5 days, isolated again, and allowed to produce an F_1 generation. The condition of egg masses and the phenotype (albino vs. wild-type pigmentation) of the progeny were noted. Since these snails may reproduce by self-fertilization, it was necessary to confirm that offspring were F_1 hybrids. The pigmentation markers previously described by Newton (1955) and Richards (1967, 1972) were used in 15 of 37 interstrain crosses. Pigmented offspring of a homozygous recessive albino parent must be F_1 hybrids. In crosses involving strains lacking pigmentation differences, it was necessary to analyse the presumed F_1 offspring electrophoretically to demonstrate their hybrid nature after they had produced sufficient F_2 progeny for genetic analysis.

The F_1 snails were individually isolated after emergence from egg masses and allowed to produce an F_2 generation by selfing. This procedure was more reliable than the traditional backcross because snails have been shown to self-fertilize some portion of their offspring even if outcrossed (Weismann, 1980). Thus in a backcross self-fertilization would confound the results.

The F_2 progeny were examined for Mendelian segregation. Random segregation of codominant alleles (electrophoretic markers) at an autosomal locus of a selfed heterozygote (i.e. A/a) should produce a ratio of 1 A/A: 2 A/a: 1 a/a among the F_2 progeny. Alternatively, for dominant traits such as the pigment marker the Mendelian model predicts a ratio of 3 pigmented:1 albino among the F_2 progeny. Often several F_1 snails from interstrain crosses were allowed to produce F_2 families.

The data for each family were examined for their fit to the predictions of the Mendelian model using the chi-square statistic (Sokal & Rohlf, 1969). Data were then pooled over all families segregating for a particular locus and an overall chi-square was calculated. Finally, a chi-square test for homogeneity was calculated to determine consistency among families.

A contingency chi-square statistic was used to test whether pairs of loci segregating in a family assorted independently. The test does not depend upon each locus being in perfect Mendelian ratios and thus is a conservative test for association. Contingency chi-square statistics were also calculated for data pooled from all families segregating for a particular pair of loci. Recombination fractions were determined by the maximum likelihood method for all pairs of loci (Allard, 1956). Recombination values were obtained by maximizing the logarithm of the probabilities of obtaining the observed genotypic distributions of families. An iterative computer program written in BASIC was used to generate these values.

RESULTS

Crossing Experiments

Thirty-seven crosses between strains of *B. glabrata* were initiated in this study (Table 2). We did not anticipate the degree of interstrain mating and genetic incompatibility that were observed within this "nominal" species, thus our culturing techniques did not permit a careful quantitative analysis of infertility and mortality resulting from these crosses. However, relevant notes regarding the mating success and the quality of egg masses are entered in Table 2. The strains used in four crosses exhibited strong reproductive in-

TABLE 2. Crosses used to examine segregation and assortment of electrophoretic and pigmentation phenotypes in *B. glabrata*. Parental strains used to initiate each cross are shown. Observations regarding crossing success are made where appropriate. Unless noted otherwise mating was apparently normal and reciprocal.

Cross	Parental strains	Observations
100	1-13-131 × Z	F_1 egg masses of Z deformed
101	M × I-10-R2	
102	M × Bz-C	
103	M × I-10-R2	Discontinued due to deaths
104	M × Bz-C	
105	M × I-10-R2	Discontinued due to deaths
106	M × Bz-C	
107	M × I-10-R2	Offspring of M not hybrids
108	M × DR	Offspring of M not hybrids
109	PF × MPR	Discontinued due to deaths
110	M × DR	Offspring of M not hybrids M egg masses deformed
111	RSF-PR1 × Z	Discontinued due to insufficient progeny
112	1-13-131 × Z	F_1 egg masses of Z deformed
113	M × DR	Discontinued due to deaths
114	1-13-131 × Z	Offspring not hybrids Z egg masses deformed
115	M × MPR	
116	Z × 963	Discontinued due to deaths
117	1-13-131 × MPR	Discontinued due to deaths
118	RSF-PR1 × 963	
119	RSF-PR1 × Bz-C	
120	L-311 × Bz-C	
121	L-311 × SCP	
122	Z × DR	Egg masses deformed
123	L-311 × 1-13-131	Discontinued due to insufficient progeny
124	L-311 × RSF-PR1	
125	RSF-PR1 × SCP	Offspring not hybrids
126	SCP × MPR	
127	DR × MPR	
128	DR × RSF-PR1	
129	M × SCP	
130	L-311 × MPR	
131	L-311 × SCP	Discontinued due to insufficient progeny
132	M × SCP	
133	M × Bz-C	
134	L-311 × MPR	
135	M × SCP	
136	1-13-131 × Z	Offspring not hybrids

compatibilities and apparent F_2 "breakdown" as evidenced by segregation distortions (Table 3). For example, crosses involving the Z strain (Brazil) were generally unsuccessful (114, 116, 136). The Z parents produced normal egg masses when selfing or when outcrossing within the Z strain, but they produced deformed egg masses (smeared and cloudy) when crossbred (between strains). The Z individuals in cross 114 produced "selfed" progeny despite the opportunity for crossbreeding. Only two out of six crosses involving the Z strain produced sufficient progeny for genetic analyses (100 and 122). Cross 122 (DR × Z) produced deformed egg masses and segregation distortions for two out of five segregating loci (Table 3).

Similarly, the DR strain (Dominican Republic) was reproductively incompatible with most other *B. glabrata* strains (Table 2). Only three of six crosses involving this strain (108, 122 and 110) produced viable F_2 progeny. In observations of matings between the M and DR strains, the DR individuals attempted to

TABLE 3. Segregation distortions in F_2 progeny of families 102, 108, 110 and 122. Expected numbers based on an assumption of 1:1 segregation of alleles are shown in parentheses. A chi-square test of the hypothesis of 1:1 segregation of alleles is given. Distorted segregation ratios are common. In crosses 108, 110 and 122, there is an excess of progeny with M-derived alleles. The last column lists the frequency of the allele generally found in excess.

F_2 of Family 102 (M/Bz-C × M/Bz-C)

	Wild		Albino	N	χ^2	Pm
Pigment	92 (103.5)		46 (34.5)	138	5.11*	0.577
	F/F	F/S	S S			
Cat	20 (24.5)	54 (49)	24 (24.5)	98	1.35	0.480
Pgm-1	33 (26.75)	55 (53.5)	19 (26.75)	107	3.88*	0.565
Me-2	27 (29.5)	65 (59)	26 (29.5)	118	1.24	0.504
Pep-2	18 (23.5)	58 (47)	18 (23.5)	94	5.14*	0.500
Est-1	31 (25.75)	52 (51.5)	20 (25.75)	103	2.35	0.553
Est-2	14 (14.5)	34 (29)	10 (14.5)	58	2.26	0.534

F_2 of Family 108 (DR/M × DR/M)

	Wild		Albino	N	χ^2	Pm
Pigment	188 (190.5)		66 (63.5)	254	0.13	0.510
	F/F	F/S	S/S			
Pgd	8 (8.5)	18 (17)	8 (8.5)	34	0.12	0.500
Ald	69 (61.5)	114 (123)	63 (61.5)	246	1.61	0.488
G6pd-1	38 (47)	100 (94)	50 (47)	188	2.30	0.532
Cat	53 (50.25)	102 (100.5)	46 (50.25)	201	0.53	0.517
Est-1	68 (52)	98 (104)	42 (52)	208	7.19**	0.563
Est-2	69 (60)	114 (120)	57 (60)	240	1.80	0.525
Est-4	44 (59.5)	125 (119)	69 (59.5)	238	5.86*	0.555
Pgm-1	25 (20.5)	42 (41)	15 (20.5)	82	2.49	0.439
Pep-2	59 (50)	95 (100)	46 (50)	200	2.19	0.533

F_2 of Family 110 (DR/M × DR/M)

	Wild		Albino	N	χ^2	Pm
Pigment	193 (201)		75 (67)	268	1.27	0.529
	F/F	F/S	S/S			
Ald	55 (59)	121 (118)	60 (59)	236	0.36	0.511
Cat	24 (30.25)	62 (60.5)	35 (30.25)	121	2.07	0.454
Est-1	63 (53.75)	112 (107.5)	40 (53.75)	215	5.30*	0.553
Est-2	69 (68.25)	134 (136.5)	70 (68.25)	273	0.10	0.498
Est-4	47 (57.25)	124 (114.5)	58 (57.25)	229	2.63	0.524
G6pd-1	34 (50)	113 (100)	53 (50)	200	6.99*	0.548
Pep-2	44 (43.75)	100 (87.5)	31 (43.75)	175	5.50*	0.537
Pgm-1	38 (41.75)	86 (83.5)	43 (41.75)	167	0.45	0.515

F_2 of Family 122 (DR/Z × DR/Z)

	F/F	F/S	S/S	N	χ^2	Pm
G6pd-1	13 (16.5)	33 (33)	20 (16.5)	66	1.48	0.553
Est-1	22 (18)	39 (36)	11 (18)	72	3.86*	0.576
Est-2	20 (19.75)	39 (36)	20 (19.75)	79	0.06	0.500
Pep-2	15 (18.75)	48 (37.5)	12 (18.75)	75	6.12*	0.520
Ald	16 (17.75)	34 (35.5)	21 (17.75)	71	0.83	0.535

inseminate M individuals but they were actively rejected when M individuals withdrew into their shells. However, several of the reciprocal matings were successful. The DR/M F_1 hybrids were viable and fertile but significant mortality in the F_2 was associated with segregation distortions (Table 3).

Mendelian Segregation

The F_2 segregation data summarized over all families for the 12 loci are presented in Table 4. When examined for data pooled over all families, the genotypic ratios at a number of loci deviated from predicted ratios. The segregation distortions for *Est-1, Est-4, Pep-2* and *G6pd-1* loci were largely, if not entirely, attributable to crosses involving the M or Z and DR strains (108, 110 and 122) and a cross involving the M and Bz-C strains (102). Chi-square statistics for an hypothesis of 1:1 segregation were recalculated without these divergent families. Without these suspect families, chi-square values for *Pig, Cat, Est-1, Est-2, Pgi, Pgd, Pgm-1* and *Ald* were within acceptable limits. Unfortunately, data for *Est-4* and *G6pd-1* were obtainable only from crosses involving the DR and M strains and they seem to reflect the same kind of segregation distortion as was observed for *Est-1* (an excess of M derived alleles; Table 3). Thus the data for *Est-4* and *G6pd-1* do not refute the Mendelian basis for these two loci.

Data for the *Me-2* locus are interesting. When examined for individual families none of the segregation ratios differed significantly from the expected ratio; however, taken together there is a significant deviation and the data are homogeneous among families. These crosses involved M and Bz-C strains and there was a slight but consistent excess of M derived alleles among the F_2 progeny. The significant homogeneity chi-square statistic associated with the *Est-2* locus reflected deviations from expected ratios but unlike *Me-2* there was no consistent direction to the deviation.

The *Pep-2* locus also exhibited significant deviations both with and without the suspect families (102, 108, 110 and 122). The deviations are due to a consistent pattern of heterozygote excess. No consistent pattern of parental alleles was found in crosses involving the *Pep-2* locus.

Linkage Analysis

Contingency chi-square statistics were calculated for all pairs of loci. Since many deviations were observed at individual loci, we chose the contingency test, since it is sensitive to the independence of the two loci and is unaffected by single locus deviations. When the null hypothesis of independence was rejected at the 0.01 level, the pair of loci were considered to be linked. Table 5 presents recombination fractions generated by the method of maximum likelihood for all loci for which pairwise tests were possible. Significant recombination fractions were underlined in Table 5. Using these values the 12 loci were assigned to one of eight provisional linkage groups (Table 6). Assignment of *Est-4* and *G6pd-1* must be viewed cautiously because they are based on crosses involving the DR and M strains.

Genetic Distance Among Strains

The genetic distances among strains based on the enzymatic loci of Mulvey & Vrijenkoek (1981a) are listed in Table 7. Among strains of *B. glabrata* the genetic distances ranged from 0.06 (M and RSF-PR1) to 0.43 (DR and 963). The DR (Dominican Republic) strain showed the greatest divergence from other *B. glabrata* strains. None of the *B. glabrata* distances was as great as those found in comparisons with *B. straminea* and *B. tenagophila.*

DISCUSSION

The development of genetic markers for the full chromosomal complement (N = 18; Burch, 1967) in *B. glabrata* is proceeding rapidly. In this study, interstrain crosses were used to examine the genetic basis for allelic isozymes encoded by eleven presumed loci. Nine of these loci (*Cat, Est-1, Est-2, Pgi, Pgd, Pep-2, Pgm-1, Me-2* and *Ald*) exhibited Mendelian segregations in most of the crosses. The segregation distortions seen for the *Est-4* and *G6pd-1* were associated with F_2 breakdown and apparent selection for "M" chromosomes in the DR/M hybrids. The results do not refute the Mendelian hypothesis for these two loci. Linkage analysis involving

TABLE 4. Segregation of alleles at polymorphic loci in F_2 progeny. Expected numbers based on an assumption of 1:1 segregation of alleles are given in parentheses. The number of families and number of individuals analysed for each locus are shown. A chi-square test, with 1 degree of freedom, for fit to a Mendelian model of 1:1 segregation is shown based on data pooled for all families and after elimination of highly divergent families. A chi-square statistic for homogeneity, with degrees of freedom equal to the number of families minus one is also given. *P ≤ 0.05, **P ≤ 0.01. For convenience, alleles at the biochemical loci are given in relative mobilities: F—Fast or S—Slow.

Locus	Genotype of progeny			Number of families	N	χ^2	χ^2_{hom}
Pigment							
	Wild	Albino					
All families	1021 (1053)	383 (351)		12	1404	3.89*	12.76
Less 102, 108, 110	640 (661.5)	242 (220.5)		9	882	2.80	7.34
	F/F	F/F	S/S				
Cat							
All families	168 (179.5)	371 (359)	179 (179.5)	9	718	1.14	9.56
Less 102, 108, 110	99 (99)	207 (198)	98 (99)	6	396	1.06	7.03
Est-1							
All families	328 (290)	598 (580)	234 (290)	11	1160	16.35*	18.31*
Less 102, 108, 110, 122	146 (139)	290 (278)	120 (139)	7	556	3.47	14.65*
Est-2							
All families	464 (453.75)	878 (907.5)	473 (453.75)	16	1815	2.01	26.19*
Less 102, 108, 110, 122	292 (289.75)	553 (579.5)	314 (289.75)	12	1159	3.26	21.29*
Est-4							
All families	94 (116.25)	244 (232.5)	127 (116.25)	2	465	5.82*	0.10
Pgi							
All families	56 (48.75)	93 (97.5)	46 (48.75)	3	195	1.44	1.04
Pgd							
All families	151 (159.5)	319 (319)	168 (159.5)	8	638	0.91	12.04
Less 108	142 (149.75)	298 (299.5)	159 (149.75)	7	599	0.41	11.99
Pep-2							
All families	156 (165.75)	369 (331.5)	138 (165.75)	7	663	9.46**	14.92*
Less 102, 108, 110, 122	20 (29.75)	68 (59.5)	31 (29.75)	3	119	4.47*	0.94
Pgm-1							
All families	228 (232.25)	485 (464.5)	211 (232.25)	10	929	2.92	16.36
Less 102, 108, 110	132 (142)	302 (284)	134 (142)	7	568	2.30	10.15
Me-2							
All families	79 (73.25)	157 (146.5)	57 (73.25)	4	293	4.81*	2.73
Less 102	52 (43.75)	92 (87.5)	31 (43.75)	3	175	5.50*	0.80
Ald							
All families	140 (138.25)	269 (276.5)	144 (138.25)	3	553	0.46	2.34
G6pd-1							
All families	85 (118)	260 (236)	127 (118)	3	472	12.36**	0.95

TABLE 5. Recombination fractions from the data pooled for all crosses (above diagonal). The number of families and the number of individuals (in parentheses) contributing to the estimate of recombination are shown below the diagonal. — test of linkage not possible with these crosses. Significant ($P \leq 0.01$) associations are underlined.

	Pig	*Ald*	*Cat*	*Est-1*	*Est-2*	*Est-4*	*G6pd-1*	*Pgd*	*Pgm-1*	*Pep-2*	*Me-2*	*Pgi*
Pig			0.49 ± .03	0.52 ± .02	0.50 ± .02	0.52 ± .03	0.50 ± .05	0.43 ± .10	0.48 ± .04	0.52 ± .05	0.44 ± .04	0.44 ± .06
Ald	2(454)		0.50 ± .03	0.49 ± .02	0.50 ± .02	0.52 ± .02	0.47 ± .07	0.47 ± .09	0.49 ± .08	0.47 ± .02	—	—
Cat	3(395)	2(296)		0.48 ± .03	0.36 ± .02	0.34 ± .03	0.46 ± .03	0.48 ± .04	0.50 ± .03	0.46 ± .03	0.47 ± .05	—
Est-1	7(810)	3(450)	3(345)		0.47 ± .02	0.37 ± .02	0.49 ± .03	0.56 ± .08	0.45 ± .02	0.57 ± .02	0.48 ± .03	0.50 ± .08
Est-2	8(915)	3(511)	5(454)	10(1024)		0.09 ± .01	0.52 ± .02	0.49 ± .03	0.48 ± .02	0.51 ± .02	0.50 ± .04	0.45 ± .06
Est-4	2(460)	2(426)	2(285)	2(417)	2(460)		0.50 ± .03	0.44 ± .09	0.47 ± .03	0.50 ± .03	—	—
G6pd-1	2(384)	3(241)	2(388)	3(388)	3(441)	2(372)		0.43 ± .10	0.45 ± .03	0.50 ± .03	—	—
Pgd	1(32)	1(32)	4(159)	2(95)	5(274)	1(30)	1(25)		0.49 ± .04	0.47 ± .06	—	0.35 ± .04
Pgm-1	8(681)	2(232)	5(316)	7(631)	9(784)	2(231)	2(211)	2(201)		0.48 ± .02	0.47 ± .03	0.44 ± .05
Pep-2	4(487)	3(395)	3(357)	6(613)	7(663)	2(345)	3(336)	2(62)	5(390)		0.52 ± .05	—
Me-2	4(278)	—	1(85)	4(219)	4(189)	—	—	—	4(238)	2(106)		—
Pgi	1(68)	—	—	1(37)	1(158)	—	—	1(106)	1(78)	—	—	

TABLE 6. Linkage groups of *B. glabrata* following maximum likelihood estimates of recombination fractions for pairs of loci.

Linkage group	Assigned loci (recombination fraction)						
I	*Est-1*	37 ± 2	*Est-4*	9 ± 1	*Est-2*	36 ± 2	*Cat*
II	*Pgd*	35 ± 5	*Pgi*				
III	*Me-2*						
IV	*Ald*						
V	*G6pd-1*						
VI	*Pig*						
VII	*Pep-2*						
VIII	*Pgm-1*						

TABLE 7. Genetic distance values (Nei, 1972) for ten strains of *Biomphalaria* used for genetic analysis and for two New World species.

	M	I-10-R2	RSF-PR1	MPR	963	SCP	Z	Bz-C	L-311	DR	*B. straminea*	*B. tenagophila*
M	—	0.15	0.06	0.08	0.11	0.08	0.14	0.10	0.17	0.30	0.96	1.34
I-10-R2		—	0.16	0.09	0.33	0.22	0.12	0.16	0.24	0.25	0.94	1.35
RSF-PR1			—	0.05	0.15	0.14	0.09	0.11	0.10	0.25	1.07	1.24
MPR				—	0.21	0.16	0.12	0.11	0.16	0.18	1.05	1.35
963					—	0.19	0.28	0.17	0.29	0.43	1.12	1.02
SCP						—	0.16	0.15	0.22	0.39	0.85	1.15
Z							—	0.19	0.14	0.24	1.01	1.30
Bz-C								—	0.27	0.25	1.01	1.19
L-311									—	0.34	0.96	1.29
PR										—	1.26	1.14
I-R-59222											—	0.92
BtBP												—

these eleven loci and the body pigment locus, *Pig* (Richards, 1973b), permit the assignment of these markers to eight linkage groups (Table 6). We plan to continue to develop these biochemical-genetic linkage maps as more strains and population samples of *B. glabrata* become available. With the morphological markers described by Richards (1973b) and an alcohol dehydrogenase locus (Narang & Narang, 1976a), it may soon be possible to have genetic markers for the 18 potential linkage groups. These markers should prove useful in formal genetic and biometric genetic studies of susceptibility and resistance to schistosomes by these snails.

The observation of linked esterase loci was not unusual. Linked esterase isozymes have previously been reported for the teleost fishes *Poeciliopsis* and *Xiphophorus* (Leslie & Pontier, 1980) and rodents, *Mus* and *Rattus* (Womach & Sharp, 1976). Esterase linkage arrangements were reflected in the observed segregation distortions. Further investigation would be required to determine whether selection is acting on these electrophoretic loci or linkage group I as a whole. Linkage disequilibria for esterases recently observed in natural populations of the snail *Cepaea nemoralis* (Selander & Foltz, 1981) might be maintained if esterase linkages similar to those of *B. glabrata* were also characteristic of *Cepaea.*

None of the genetic distances between strains of *B. glabrata* was as great as those between *B. glabrata* and either *B. straminea* or *B. tenagophila* (Table 8). Nevertheless, the DR (Dominican Republic) strain is clearly the most divergent *B. glabrata* strain given the enzymatic markers we examined (Nei's D = 0.43). Goldman *et al.* (1984) have shown that the DR strain differs from other *B. glabrata* in the number of metacentric chromosomes. It exhibits the classical features typically attributed to semispecies of *Drosophila*, ". . . populations which have part way completed the process of speciation. Gene exchange is still

possible among semispecies but not as freely as among conspecific populations" (Mayr, 1963: 501). Insemination attempts by the DR strain were consistently rejected by the NIH-albino M strain, but the reciprocal matings did occur. The DR/M hybrids exhibited semisterility (deformed egg masses and low fecundity) and the progeny suffered high mortality. Segregation distortions occurred for several loci in these hybrids and consistently favored the M alleles associated with linkage group I (Table 3). Interestingly, not all linkage groups showed significant distortion although there was an overall excess of M alleles for most loci. The Z strain (Brazil) also was difficult to cross with other strains but it did not reflect the degree of genetic distance (D range: 0.09 to 0.28) found with the DR strain. In a cross with the DR strain (122: DR/Z) the Z alleles were favored in the segregation distortions (Table 3).

Our mating experiments between strains of *B. glabrata* revealed incompatibilities with respect to mate preference and egg production and survivorship. Paraense (1959) also reported reproductive isolation between geographically remote populations of *B. glabrata.* Laboratory strains of *B. glabrata* may represent a similar phenomenon since they frequently originated in distant areas of the species range and have been isolated in the laboratory for many generations. Erhman (1969) and Powell (1978) reported development of sexual isolation among laboratory populations of *Drosophila pseudoobscura* in the absence of selection and as a by-product of genetic differentiation and genetic drift during isolation.

The consistent segregation distortions in the F_2 progeny of crosses involving the DR and M strains of *B. glabrata* have been attributed to genetic incompatibility. As discussed by Kitagawa (1967) the fitness of hybrids between populations or races is not predictable because coadapted gene complexes seem to be a feature of populations and not of species. Successful F_1 hybrids and subsequent breakdown in F_2 or later generations has frequently been reported (Wallace & Vetukhiv, 1955; Kitagawa, 1967; Anderson, 1968). Considering the broad geographical distribution of *B. glabrata*, environmental variability might lead to geographical differentiation with local populations having their own coadapted gene complexes. The strains having relatively large genetic distance values, as measured by electrophoresis, also were observed to result in the production of deformed, milky egg masses and suggest genomic incompatibility in F_2 breakdown. A determination of how much of the apparent reproductive isolation observed in the present study may be a by-product of laboratory isolation and how much reflects naturally occurring reproductive barriers must await further study.

The present evidence for divergence among geographical strains of *B. glabrata* is consistent with the hypothesis that natural populations of these snails are highly subdivided and often exhibit a patchy distribution of susceptibility to schistosomes. Michelson & Dubois (1978) proposed that this patchiness was due to genetic drift. We attempted to test this hypothesis with our electrophoretic markers (Mulvey & Vrijenhoek, 1982). Although we do not contend that these markers are directly involved with resistance or even are closely linked with resistance genes, the genetic data presented here do suggest that they mark a reasonable portion of the snail's genome. The *B. glabrata* populations in Puerto Rico were found to be highly subdivided genetically, suggesting a history of genetic drift and low interdrainage migration. Research in progress with *B. alexandrina* from Egypt reveals significantly less differentiation within the lower Nile drainage, including Aswan (Graven & Vrijenhoek, 1983). These studies and a breeding system study of *B. obstructa* from Florida all revealed that inbreeding and "selfing" are not occurring to a significant degree in natural populations of *Biomphalaria* (Mulvey & Vrijenhoek, 1981b). The genetic markers developed in our formal genetic studies should continue to prove useful in studies of the population genetics and systematic relationships of *Biomphalaria,* as well as providing a tool for the genetic analysis of schistosome resistance and susceptibility.

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LITERATURE CITED

ALLARD, R. W., 1956, Formulas and tables to facilitate the calculation of recombination values in heredity. *Hilgardia,* 24: 235–278.

ANDERSON, W. W., 1968, Further evidence of coadaptation in crosses between geographic populations of *Drosophila pseudoobscura.* IV. Relative viability. *Genetical Research,* 10: 317–330.

BURCH, J. B., 1967, Chromosomes of intermediate hosts of human bilharziasis. *Malacologia,* 5: 127–135.

ERHMAN, L., 1969, Genetic divergence in M. Vetukhiv's experimental populations of *Drosophila pseudoobscura.* 5. A further study of rudiments of sexual isolation. *American Midland Naturalist,* 82: 272–276.

GOLDMAN, M. A., LoVERDE, P. T., CHRISMAN, C. L. & FRANKLIN, D. A., 1984, Chromosomal evolution in planorbid snails of the genera *Bulinus* and *Biomphalaria. Malacologia,* 25: 427–446.

GRAVEN, M. A. & VRIJENHOEK, R. C., 1983, Population structure of Egyptian *Biomphalaria alexandrina.* Meeting of the American Society of Tropical Medicine and Hygiene, San Antonio, 5–8 December 1983. Abstract.

HENDRICKSEN, U. B. & JELNES, J. E., 1980, Experimental taxonomy of *Biomphalaria* (Gastropoda: Planorbidae). I. Methods of experimental taxonomic studies of *Biomphalaria* carried out by horizontal starch gel electrophoresis and staining of twelve enzymes. *Journal of Chromatography,* 188: 169–176.

JELNES, J. E., 1982, Experimental taxonomy of *Biomphalaria* (Gastropoda: Pulmonata). II. Electrophoretic observations on eight enzyme systems of the South American species: *Biomphalaria glabrata, B. straminea* and *B. tenagophila. Journal of Natural History,* 16: 209–217.

KITAGAWA, O., 1967, Genetic divergence in M. Vetukhiv's experimental populations of *Drosophila pseudoobscura.* IV. Relative viability. *Genetical Research,* 10: 303–312.

LESLIE, J. F. & PONTIER, P. J., 1980, Linkage conservation of homologous esterase loci in fish (Cyprinodontoidea: Poecilidae). *Biochemical Genetics,* 18: 103–115.

MAYR, E., 1963, *Animal species and evolution.* Harvard University, Cambridge, Mass, 501 p.

MICHELSON, E. A. & DuBOIS, L., 1978, Susceptibility of Bahian populations of *Biomphalaria glabrata* to an allopatric strain of *Schistosoma mansoni. American Journal of Tropical Medicine and Hygiene,* 27: 782–786.

MONTIERO, W. & NARANG, S., 1976, Genetic variation in two natural populations of *Biomphalaria tenagophila:* Esterases and alcohol dehydrogenase. *Anais da Academia Brasileira de Ciencias,* 48: 341–346.

MULVEY, M. & VRIJENHOEK, R. C., 1981a, Genetic variation among laboratory strains of the planorbid snail *Biomphalaria glabrata. Biochemical Genetics,* 19: 1169–1182.

MULVEY, M. & VRIJENHOEK, R. C., 1981b, Multiple paternity in the hermaphroditic snail, *Biomphalaria obstructa. Journal of Heredity,* 72: 308–312.

MULVEY, M. & VRIJENHOEK, R. C., 1982, Population structure in *Biomphalaria glabrata:* examination of an hypothesis for the patchy distribution of susceptibility to schistosomes. *American Journal of Tropical Medicine and Hygiene,* 31: 1195–1200.

NARANG, S. & NARANG, N., 1974, Characterization of malate dehydrogenase: electrophoresis, isoelectric focusing, thermostability, inhibition and activity studies on homogenates of various organs of *Biomphalaria glabrata* (Mollusca: Pulmonata). *Comparative Biochemistry and Physiology,* 48B: 477–490.

NARANG, S. & NARANG, N., 1976a, Alcohol dehydrogenase of *Biomphalaria glabrata* (Mollusca: Pulmonata). Polymorphism, genetic analysis and interspecific variations. *Biochemical Genetics,* 14: 75–84.

NARANG, S. & NARANG, N., 1976b, Esterases in *Biomphalaria glabrata* (Mollusca: Pulmonata) characterization, polymorphism, inheritance pattern and interspecies differences. *Anais da Academia Brasileira de Ciencias,* 48: 255–262.

NARANG, S. & NARANG, N., 1976c, Glucose-6-phosphate dehydrogenase of *Biomphalaria glabrata* (Say 1818) (Mollusca: Pulmonata). Characterization and inheritance pattern. *Anais da Academia Brasileira de Ciencias,* 48: 125–131.

NEI, M., 1972, Genetic distance between populations. *American Naturalist,* 106: 283–292.

NEI, M., 1978, Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics,* 89: 583–590.

NEWTON, W. L., 1953, The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabratus. Experimental Parasitology,* 41: 242–257.

NEWTON, W. L., 1955, The establishment of a strain of *Australorbis glabratus* which combines albinism and high susceptibility to infection with *Schistosoma mansoni. Journal of Parasitology,* 41: 526–528.

PARAENSE, W. L., 1959, One-sided reproductive isolation between geographically remote populations of a planorbid snail. *American Naturalist,* 93: 93–101.

POWELL, J. R., 1978, The Founder-Flush speciation theory: an experimental approach. *Evolution,* 32: 465–474.

RICHARDS, C. S., 1967, Genetic studies on *Biomphalaria glabrata* (Basommatophora: Planorbi-

dae), a third pigmentation allele. *Malacologia,* 5: 335–340.

RICHARDS, C. S., 1972, Pigmentation variations in *Biomphalaria glabrata* and other Planorbidae. *Malacological Review,* 6: 49–51.

RICHARDS, C. S., 1973a, Susceptibility of adult *Biomphalaria glabrata* to *Schistosoma mansoni* infection. *American Journal of Tropical Medicine and Hygiene,* 22: 748–756.

RICHARDS, C. S., 1973b, Genetics of *Biomphalaria glabrata* (Gastropoda: Planorbidae). *Malacological Review,* 6: 199–202.

RICHARDS, C. S., 1975a, Genetic studies on variation in infectivity of *Schistosoma mansoni. Journal of Parasitology,* 61: 233–236.

RICHARDS, C. S., 1975b, Genetic factors in susceptibility of *Biomphalaria glabrata* for different strains of *Schistosoma mansoni. Parasitology,* 70: 231–241.

RICHARDS, C. S., 1976a, Variations in infectivity for *Biomphalaria glabrata* in strains of *Schistosoma mansoni* from the same geographical area. *Bulletin of the World Health Organization,* 54: 706–707.

RICHARDS, C. S., 1976b, Genetics of the host-parasite relationship between *Biomphalaria glabrata* and *Schistosoma mansoni.* In TAYLOR, A. E. R. & MULLER, R., ed., *Genetic Aspects of Host-Parasite Relationships,* p. 45–54. Blackwell Scientific, Oxford.

RICHARDS, C. S. & MERRITT, J. W., 1972, Genetic factors in the susceptibility of juvenile *Biomphalaria glabrata* to *Schistosoma mansoni infection. American Journal of Tropical Medicine and Hygiene,* 21: 425–434.

SAOUD, F. F. A., 1965, Susceptibility of various snail intermediate hosts of *Schistosoma mansoni* to different strains of the parasite. *Journal of Parasitology,* 39: 363–376.

SELANDER, R. K. & FOLTZ, D. W., 1981, Genetic disequilibrium between esterase loci in populations of *Cepaea nemoralis* in Western New York. *Evolution,* 35: 190–191.

SOKAL, R. R. & ROHLF, F. J., 1969, *Biometry.* Freeman, San Francisco, p. 561–566.

WALLACE, B. & VETUKHIV, M., 1955, Adaptive organization of the gene pools of *Drosophila* populations. *Cold Spring Harbor Symposium,* 20: 303–310.

WEISMANN, D., 1980, Selfing and outcrossing in the snail *Biomphalaria glabrata.* Unpublished Henry Rutgers Honors Thesis, Rutgers University, New Jersey, 31 p.

WOMACH, J. E. & SHARP, M., 1976, Comparative autosomal linkage in mammals: Genetics of esterases in *Mus musculus* and *Rattus norvegicus. Genetics,* 82: 665–675.

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GENETICS OF THE CLAM *MERCENARIA MERCENARIA*. II. SIZE AND GENOTYPE

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ABSTRACT

This paper reports on the genetic structure of a population of *Mercenaria mercenaria* bred and reared under hatchery conditions. We also report on the relationship between allozyme genotypes at five loci and shell size at the age of one year. Clams of known genotype, from a wild population of known composition, were individually induced to spawn. All gametes were mixed at one time to produce a randomly bred cohort of clams. After one year, 1081 of these clams were measured and their phenotypes determined by starch gel electrophoresis for the enzymes *Pgd, Lap, Pgi, Pgm-2* and *Pgm-3*.

The cohort's genotype frequencies differed from Hardy-Weinberg expectation for all genes except *Pgd* and *Pgm-2*, but only for *Lap* was the deviation associated with heterozygosity, for which there was a striking deficiency. Since the cohort was randomly bred, the Wahlund effect cannot explain this observation while differential survival can. The joint frequencies by pairs for three loci (*Lap, Pgm-3, Pgi*) were significantly different from expectations based on independence. Because these three loci assort independently in Mendelian crosses, this lack of independence suggests interaction effects on survival. Two genes, *Lap* and *Pgm-3*, show highly significant associations of genotype with shell size. In the case of *Pgm-3*, the data fit a model of additive allele effects with no interaction. For *Lap*, allelic interactions do occur in certain genotypes. In neither instance is there a specific effect of heterozygosity *per se*.

Key words: clam; *Mercenaria*; genetics; allozyme; LAP; growth.

INTRODUCTION

Extensive studies of genes encoding enzymes have been made on natural populations of marine bivalves. Koehn *et al.* (1973, 1976) and Milkman & Koehn (1977) have reported widespread deficiencies of heterozygotes for the gene *Lap* in the mussels *Mytilus edulis* and *Modiolus demissus* and their work supports the hypothesis that selection causes these deficiencies. Other workers have reported this pattern of deficient heterozygotes for additional allozyme loci in natural populations of other bivalves such as oysters (Zouros *et al.*, 1980) and *Mercenaria* (Humphrey, 1982). All of these studies deal with wild-caught individuals whose parental population is unknown. This uncertainty about the origin of sedentary adults always complicates the interpretation of data on genotype frequencies in organisms with a pelagic larval stage because the possibilities of Wahlund's effect (i.e. population mixing) and of nonrandom mating cannot usually be excluded. To control these factors, we have studied genotype frequencies in a closed, laboratory bred population of the hard clam *Mercenaria mercenaria* (Linnaeus, 1758) (Veneridae).

Because our population has a uniform age, we can also examine the effects of allozyme loci on shell length without confounding age and size. Koehn and his colleagues, as cited above, have shown an association between genotype and size in natural populations of mussels and Zouros *et al.* (1980) have reported an association between size and heterozygosity in oysters. Newkirk and his colleagues (Newkirk, 1978; Newkirk *et al.*, 1977; Innes & Haley, 1977) have investigated several continuous traits in mussels and oysters using strictly quantitative techniques without identifying individual loci. In this paper we show that allozyme genotypes have demonstrable effects on shell size in *M. mercenaria* and that these effects can be described with quantitative models containing estimable parameters.

MATERIALS AND METHODS

We have studied genes coding for the enzymes phosphoglucomutase II and III (ECC 2.7.5.1, *Pgm-2* and *Pgm-3*), phosphoglucose isomerase (ECC 5.3.1.9, *Pgi*), leucine aminopeptidase (ECC 3.4.1.1, *Lap*) and 6-phosphogluconate dehydrogenase (ECC

1.1.1.43, *Pgd*). *Pgd* was added rather late in the study and little information is available on it compared to the other four genes. All enzymes were resolved by starch gel electrophoresis using methods described elsewhere (Adamkewicz *et al.*, 1984).

Samples of three natural populations have been surveyed for these genes and will be described more extensively elsewhere. The artificial clam population designated Mixed Spawn was bred and reared at the Virginia Institute of Marine Science (VIMS) Eastern Shore Laboratory at Wachapreague. Wild caught clams from Burton's Bay were brought to the hatchery and induced to spawn by a combination of heat shock and exposure to killed sperm (Castagna & Kraeuter, 1981). Eggs and sperm were collected separately and, when spawning ceased, the pooled batches of eggs were mixed with the pooled batches of sperm to produce the Mixed Spawn zygotes. The larvae were reared to settling (10 to 14 days in *M. mercenaria*) in a vat with enriched sea water. After settling, the clams were transferred to a flow-through sea table and allowed to grow for one year.

Of 110 wild clams treated, 44 spawned (23 males and 21 females). No attempt was made to estimate the contribution of each parent. As a result, the genotypic frequencies of Mixed Spawn cannot be predicted on the basis of parental genotypes although these are known. The conditions under which the eggs and sperm were mixed did assure the opportunity for random mating and the results of two Mendelian crosses (Adamkewicz *et al.*, 1984) have shown that union of gametes is indeed random. Therefore, the individuals of Mixed Spawn began life as a randomly bred cohort. Survival from fertilization to the end of the first year was estimated to be one per thousand.

This paper describes studies on a sample of about 1,100 clams taken from the Mixed Spawn population at age one year. To obtain a random sample, the entire population of about 22,000 clams was washed, heaped into a single pile and divided into sectors. The sample was bagged and stored at $-70°C$ until tested. The remaining clams were grown for a second year and will be reported on at a later date.

Before each clam was examined electrophoretically, maximum shell length was measured to the nearest 0.1 mm. Lengths were transformed logarithmically before statistical analysis. The appropriateness of this transformation was confirmed by examining the distribution of residual $\log_{10}$ (length), that is the distribution of each observation minus its genotype mean. This variable was normally distributed (Kolmogorov-Smirnov test, $p > 0.15$).

To reduce the number of genotypes in our analyses, very rare alleles (frequency $\leqslant 0.005$) were eliminated by discarding any individual carrying them. This was done for *Pgd* (3 of 162), *Pgm-2* (7 of 1070), *Pgm-3* (7 of 1067) and *Pgi* (7 of 1061). In the case of *Pgi,* genotypes were merged by combining alleles Pgi^3 (frequency 0.011) and Pgi^5 (frequency 0.026) with Pgi^4 (frequency 0.047). These alleles have very similar electrophoretic mobilities and are subject to some scoring error. Analyses were performed on both altered and original data. In no case did alteration change a conclusion. The data for *Lap* were not altered in any way.

The effects of individual alleles on length were estimated with a model of additive allele effects. The model is a multiple linear regression equation

$$\mu_y = \beta_o + \sum_{i=i}^{A-1} \beta_i X_i$$

where μ_y equals the mean length of genotype y, A is the number of alleles at the locus under study, the β_i are the individual allele effects, and the X_i are indicator variables which are defined to reflect zero, one or two doses of the i^{th} allele (*i.e.* to represent genotype) and whose values are subject to constraints which result in

$$\sum_{i=1}^{A} \beta_i = 0$$

A model that allows all possible allelic interactions (dominance, overdominance, *etc.*) is

$$\mu_y = \beta_o + \sum_{i=1}^{A-1} \beta_i X_i + \sum_{i=1}^{A} \sum_{j=1}^{A} \gamma_{ij} X_{ij}$$

where the γ_{ij} are interaction terms and

$$X_{ij} = 0 \text{ for } i \geqslant j$$

$$X_{ij} = 1 \text{ for } i < j.$$

The parameters of these models were estimated using the General Linear Models procedure of SAS 1979.5 (SAS Institute, Cary, N.C.).

RESULTS

Genotype frequencies. Of the five loci studied in the Mixed Spawn populations, two (*Pgd* and *Pgm-2*) did not differ significantly from Hardy-Weinberg expectations. The other three loci (*Lap, Pgi* and *Pgm-3*) did deviate significantly as shown in Tables 1, 2 and 3. Because exact parental contributions are not known, these Hardy-Weinberg expectations are based on observed allele frequencies in Mixed Spawn itself. However, comparisons to expectations based on equal contributions by all parents were also made. If the genotype frequencies in Mixed Spawn had been those expected from the male and female parents' allele frequencies, they would have conformed very closely to Hardy-Weinberg expectations at each locus. Genotype frequencies for *Pgm-2* did in fact conform to parental expectations ($p > 0.3$) and to Hardy-Weinberg ($p = 0.32$). This finding suggests that parental contributions were approximately equal and that the deviations observed for *Lap, Pgm-3,* and *Pgi* were caused by differential viability among genotypes.

The deviation of *Lap* (Table 1) shows a pattern distinctly different from that of *Pgm-3* (Table 2) and *Pgi* (Table 3). For *Lap,* every heterozygote except $Lap^{3/5}$ is deficient. For *Pgm-3* and *Pgi,* the heterozygotes show deviations in both directions, excesses as well as deficiencies. *Pgm-3* and *Pgi* have only one common homozygote, also their commonest genotype. In both cases, the frequency of this homozygote is very close to expectation. *Lap* has four homozygotes, all in excess.

Lap also shows a pattern different from *Pgm-3* and *Pgi* when genotype frequencies of Mixed Spawn are compared to those in natural populations. Three natural populations have statistically indistinguishable genotype frequencies for the *Lap, Pgm-3* and *Pgi* loci. The observed and expected genotype frequencies for these three natural populations, combined into a single population for comparison to Mixed Spawn, are shown in Tables 1 through 3. Unlike Mixed Spawn, the natural populations do not differ from Hardy-Weinberg expectation for the loci *Pgm-3* and *Pgi.* Like Mixed Spawn, the combined population does differ significantly from Hardy-Weinberg for *Lap.* For all three loci, the genotype frequencies of Mixed Spawn are significantly different from those of the natural populations. How much of this difference may be due to selection for survival under very artificial conditions we cannot say. Almost certainly, the differences of Mixed Spawn from Hardy-Weinberg and from parental expectations are due to selection. The differences between Mixed Spawn and the natural populations might, however, be caused by founder effects or by a difference in the amount of time during which selection could act (the clams from the natural populations were estimated to be five or more years old).

For *Lap,* more information is available. Although the samples from the three natural populations have statistically indistinguish-

TABLE 1. Observed distribution of *Lap* genotypes in Mixed Spawn and in natural populations. The distribution expected for Hardy-Weinberg is also shown and p{HW} is the probability that the observed values conform to expectations. Genotypes are listed in order of decreasing average shell length as measured in Mixed Spawn. The probability that the Mixed Spawn and natural populations are alike is 0.0001.

	Mixed spawn		Natural populations	
Lap genotype	Observed	Expected	Observed	Expected
3/5	0.026	0.016	0.009	0.004
3/4	0.351	0.391	0.345	0.336
4/4	0.191	0.153	0.113	0.112
3/3	0.272	0.250	0.267	0.251
2/3	0.078	0.092	0.115	0.159
2/2	0.029	0.008	0.052	0.025
2/4	0.048	0.072	0.098	0.106
2/5	0.002	0.002	0	0.001
5/5	0.001	0.000	0	0.000
4/5	0.003	0.013	0	0.003
N	1048		461	
p{HW}	0.0001		0.0001	

TABLE 2. Observed distribution of *Pgm-3* genotypes in Mixed Spawn and in natural populations. The distribution expected for Hardy-Weinberg is also shown and p{HW} is the probability that the observed values conform to expectation. Genotypes are listed in order of decreasing average shell length as measured in Mixed Spawn. The probability that the Mixed Spawn and natural populations are alike is 0.0001.

	Mixed spawn		Natural populations	
Pgm genotype	Observed	Expected	Observed	Expected
3/3	0.003	0.002	0.014	0.002
2/3	0.054	0.063	0.053	0.067
2/2	0.509	0.504	0.487	0.466
2/7	0.096	0.090	0.142	0.146
3/4	0.023	0.016	0.014	0.017
3/7	0.007	0.006	0.005	0.010
2/2	0.251	0.259	0.230	0.235
7/7	0.006	0.004	0.018	0.012
4/4	0.040	0.033	0	0.000
4/7	0.012	0.023	0.037	0.037
N	1060		431	
p{HW}	0.02		0.36	

TABLE 3. Observed distribution of *Pgi* genotypes in Mixed Spawn and in natural populations. The distribution expected for Hardy-Weinberg is also shown and p{HW} is the probability that the observed values conform to expectation. Genotypes are listed in order of decreasing average shell length as measured in Mixed Spawn. The probability that the Mixed Spawn and natural populations are alike is 0.0056.

	Mixed spawn		Natural populations	
Pgi genotype	Observed	Expected	Observed	Expected
1/7	0.001	0.004	0.002	0.002
1/1	0.002	0.004	0	0.002
1/4	0.016	0.011	0.012	0.005
4/4	0.010	0.007	0.004	0.004
2/4	0.002	0.005	0.002	0.004
4/7	0.004	0.005	0.004	0.003
4/6	0.126	0.134	0.099	0.106
6/6	0.630	0.626	0.720	0.716
1/6	0.097	0.103	0.065	0.066
1/2	0.012	0.004	0	0.003
6/7	0.059	0.050	0.032	0.035
2/6	0.041	0.044	0.056	0.055
2/7	0	0.002	0.002	0.001
7/7	0	0.001	0	0.000
2/2	0	0.001	0.002	0.001
N	1054		464	
p{HW}	0.001		0.42	

able genotype frequencies at this locus, the samples from populations I and II do show the same deficiency of heterozygotes seen in Mixed Spawn. The sample from population III does not (Adamkewicz *et al.*, unpublished). Because the sample of population III was taken from the area of the hatchery's water source, its members lived under a regimen of temperature and salinity very like that of Mixed Spawn. This similarity in water quality has not resulted in a similarity in genotype frequencies.

The frequency distributions of two-locus genotypes were also investigated. Each of

the ten pair-wise combinations of the five loci was examined for independence by a chi squared test. The combined natural populations showed no evidence of association between loci. The Mixed Spawn clearly did. The frequencies of three pairs (*Lap/Pgi, Lap Pgm-3, Pgm-3/Pgi*) differed significantly from their joint expectation based on the hypothesis of independence. Results of controlled matings have shown that these three loci assort independently (Adamkewicz *et al.*, 1984). Chromosomal linkage cannot, therefore, account for the deviations observed. Joint allele frequencies in the parents do not show gametic phase disequilibrium. If parental contributions were nearly equal, gametic frequencies could not account for the deviations observed. These considerations make selection the likeliest explanation for the observed departures from independence.

Table 4 displays the observed joint frequencies for paired loci and the frequencies expected if the loci were independent. For brevity's sake, only common genotypes are included. Chi squared values are highly significant and a careful examination of the complete tables shows that empty cells and those with low expectations do not contribute disproportionately to these values. The most important sources of deviation from independence are identified in the table with asterisks. These genotypes are not the principal sources of deviation from Hardy-Weinberg (Tables 1, 2, 3). Furthermore, these genotypes are not extreme in size (Tables 6, 7). The departures from independence are quite specific to particular genotypes and these departures suggest epistatic effects on survival.

Size and genotype. Fig. 1 shows the distribution of shell length in the Mixed Spawn population. The hypothesis that enzyme genotype affects size through simple overdominance has been suggested by Zouros *et al.* (1980). They showed that, in oysters of uniform age, an observed deficiency of heterozytes ($D = (H_o - H_e)/H_e$ where H_o and H_e are observed and expected heterozygosities)

TABLE 4. Numbers observed (expected) for selected two locus genotypes of *Pgi, Pgm-3* and *Lap.* Expectations are based on the assumption of independence. Those genotypes identified with an * are the most important sources of deviation from expectation.

		Lap genotype			
		3/3	*3/4*	*4/4**	
	6/6	183	254	95	
Pgi		(177.1)	(226.9)	(124.8)	
genotype	*4/6*	38	46	22	$\chi^2 = 239$, df = 99
		(35.5)	(45.5)	(25.0)	p = 0.0001
	1/6	27	21	26	
		(27.1)	(34.7)	(19.1)	
	*1/2**	1	1	11	
		(3.6)	(4.6)	(2.5)	
		Pgi genotype			
		6/6	*4/6**	*1/6*	
	2/2	368	47	52	
Pgm-3		(333.8)	(67.3)	(52.0)	
genotype	*2/3**	8	21	4	$\chi^2 = 380$, df = 99
		(35.8)	(7.2)	(5.2)	p = 0.0001
	2/4	182	39	18	
		(163.1)	(32.9)	(25.4)	
	2/7	55	9	12	
		(62.7)	(12.6)	(9.8)	
		Lap genotype			
		*3/3**	*3/4*	*4/4*	
	2/2	134	201	97	
Pgm-3		(144.3)	(187.8)	(98.7)	
genotype	*2/4*	90	92	43	$\chi^2 = 216$, df = 81
		(70.4)	(91.6)	(48.2)	p = 0.0001
	*2/7**	14	33	31	
		(27.0)	(35.1)	(18.5)	

decreased with increasing size class. To test whether size is related to heterozygosity in *M. mercenaria*, we compared the mean sizes of heterozygotes and homozygotes for each locus using Student's *t* test. No significant differences were found. Because *Lap* showed a general deficiency of heterozygotes, we carried out an analysis similar to that of Zouros for that locus: the Mixed Spawn population was divided into decile size classes and $D = (H_o - H_e)/H_e$ was calculated for each decile. The results are shown in Table 5. D is not correlated with size class nor is any trend apparent.

TABLE 5. Relative deviation of heterozygotes by decile size class at the *Lap* locus in Mixed Spawn. D is calculated as in Zouros *et al.* (1980) to equal $(H_o - H_e)/H_e$. The probability given is that associated with a chi squared test of observed versus expected heterozygotes.

Decile	D	Probability
1	−0.183	<0.05
2	−0.074	ns
3	−0.236	<0.05
4	−0.118	ns
5	−0.110	ns
6	−0.216	<0.05
7	−0.083	ns
8	−0.143	ns
9	−0.242	<0.05
10	+0.141	ns

To investigate the possibility that size is related to the total amount of heterozygosity over four loci (*Pgd* was excluded due to small sample size), each clam was assigned a score from 0 to 4 according to the number of loci at which it was heterozygous. An analysis of variance comparing mean size within score classes showed no significant effect of heterozygosity score.

An effect of genotype on size can, however, be demonstrated. An analysis of variance of size within genotype for each locus showed a significant effect of genotype on size for *Lap* ($P = 0.0008$) and for *Pgm-3* ($p = 0.0027$). Tables 6 and 7 present the mean shell length for each genotype for *Lap* and *Pgm-3*. The simplest model to account for the effect of genotype on size is one of additive allele effects. The data for *Pgm-3* (Table 6) certainly suggest that such a model is valid: all of the *Pgm-3* heterozygotes (except $Pgm\text{-}3^{4/7}$) have means inside the range of their homozygotes. An indicator variable analysis, with no interaction terms (dominance, etc.), was used, as described in the methods section, to estimate the additive effect of each allele on size. The estimated allele effects then were used to predict mean size for each genotype. As Table 6 shows, this model fits the data for

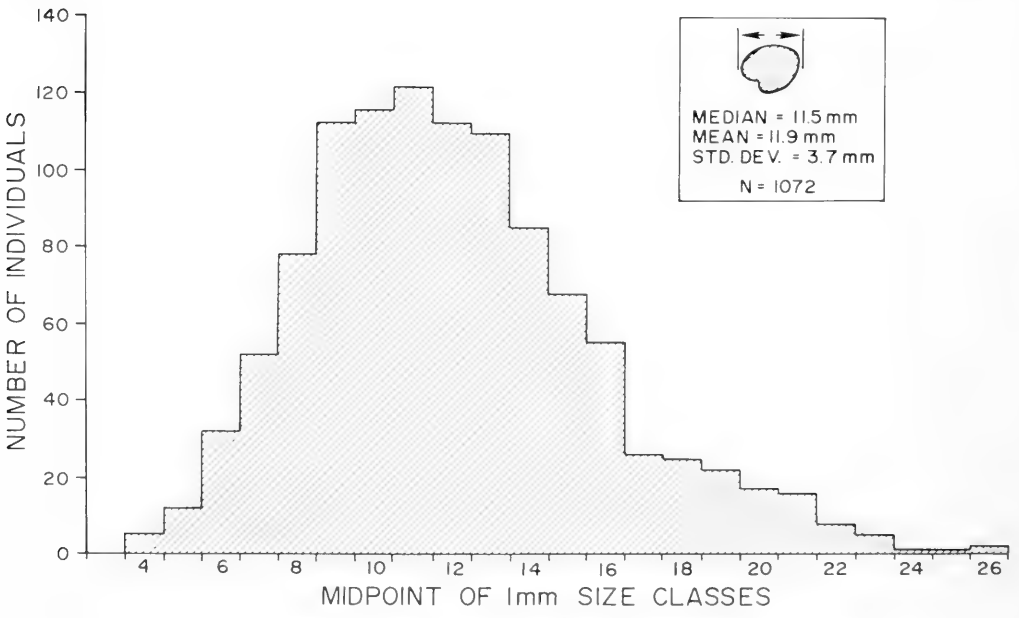

FIG. 1. Distribution of shell length in one year old *Mercenaria mercenaria*.

TABLE 6. Observed mean shell length by *Pgm-3* genotype compared to the shell length predicted by a model of additive allele effects with no interaction. Measurements are for 1060 individuals of Mixed Spawn.

Pgm-3 genotype	N	Mean length mm ± SE of mean		Predicted length mm
3/3	3	16.1	2.8	13.2
2/3	57	12.7	0.5	12.8
2/2	540	12.3	0.2	12.3
2/7	102	12.0	0.3	11.8
3/4	24	11.6	0.6	11.8
2/4	266	11.5	0.2	11.4
3/7	7	11.3	0.9	12.2
7/7	6	10.4	1.6	11.2
4/4	42	10.4	0.6	10.5
4/7	13	10.0	0.5	10.8

TABLE 7. Observed mean shell length by *Lap* genotype compared to the shell length predicted by a model of additive allele effects with no interactions. Measurements are for 1048 individuals of Mixed Spawn.

Lap genotype	N	Mean length mm ± SE of mean		Predicted length mm
3/5	27	12.7	0.5	11.9
3/4	371	12.5	0.2	12.1
4/4	200	12.1	0.2	12.3
3/3	286	11.6	0.2	11.9
2/3	82	11.1	0.3	11.2
2/2	30	11.0	0.6	10.4
2/4	51	10.8	0.6	11.4
2/5	2	9.6	0.1	11.2
5/5	1	8.7	—	11.9
4/5	3	8.3	1.7	12.1

Pgm-3. The predicted means show close correspondence to the observed means and only two genotypes, $Pgm\text{-}3^{7/7}$ and $Pgm\text{-}3^{4/7}$, are ranked differently in the predicted series than in the observed series.

On the other hand, a simple additive model does not fit the data for *Lap* (Table 7). Only two ($Lap^{2/3}$ and $Lap^{2/5}$) of the six heterozygotes have observed mean sizes inside the range of their homozygotes. Exploration of the full model using maximum R^2 stepwise regression shows that successful *Lap* models must include allelic interaction terms. For this locus, the Lap^3-Lap^4 interaction term is more important than any of the estimated additive allele effects in predicting mean shell length. We note, however, that over-dominance *per se* is not a useful explanation of these results. Not only are some heterozygotes observed to be intermediate to their homozygotes, the $Lap^{2/4}$ and $Lap^{4/5}$ heterozygotes are actually observed to be smaller than the smallest of their two homozygotes. The effect of the *Lap* locus on shell size clearly is genotype specific and it involves allele specific interaction of several different kinds.

DISCUSSION

The genotype frequencies in the Mixed Spawn population are evidence of strong selection on the *Lap* locus. Koehn *et al.* (1973, 1976) and Milkman & Koehn (1977) have argued cogently for this same conclusion regarding *Lap* in the mussels *Mytilus edulis* and *Modiolus demissus*. Natural populations of both these species show the same deficiency of heterozygotes seen in the Mixed Spawn clams. Because of Mixed Spawn's artificial origin, two other potential causes of this heterozygous deficiency can be eliminated with certainty. Wahlund's effect, the pooling of heterogeneous populations, cannot occur because Mixed Spawn is a closed population. Inbreeding cannot be the cause of the

deficiency because the Mixed Spawn clams were randomly bred. Marked allele frequency differences between male and female parents cannot be excluded as a cause of the observed deficiency but this factor is unlikely to be acting given the evidence from the *Pgm-2* locus. The presence of null alleles, leading to misidentification of heterozygotes as homozygotes, is another cause that cannot be excluded with certainty. However, Koehn *et al.* (1976) and Zouros *et al.* (1980) have already shown that such an explanation requires an unreasonably high frequency of null alleles. Only selection through differential viability remains as a reasonable explanation of our findings. Our data cannot exclude the possibility that selection acts on closely linked loci rather than on *Lap* itself. However, since deficiencies of *Lap* heterozygotes occur in natural populations of *M. mercenaria* and many other marine pelecypods, selection is much more likely to be acting directly on the *Lap* locus.

We can begin to identify the times in the life cycle at which this selection must be acting. Clams from controlled crosses were raised for five months under the same conditions that the Mixed Spawn clams were subjected to for twelve months. The five month old clams showed no deficiency or excess of any *Lap* genotype (Adamkewicz *et al.,* 1984). This observation suggests strongly that selection against heterozygotes occurs in the second half of the first year of life. Selection against heterozygotes leads to unstable allele frequencies unless the selection is frequency dependent. *Lap* allele frequencies in adult *M. mercenaria* appear much the same throughout the species' range (Humphrey,1982), suggesting that the polymorphism is in fact a stable one. No evidence exists at present for frequency dependent effects in oysters (Zouros *et al.,* 1980) or in the Mixed Spawn clams. We found no relationship between frequency and either size or departure from Hardy-Weinberg expectation. Net selection over the entire life cycle must, therefore, be very different from the selection found in one year old clams if the polymorphism is to be stable. The possibility of selection via differential reproduction is under investigation in *Mercenaria.* Zouros & Foltz (1984) have suggested that heterozygous advantage at reproduction may be common in marine bivalves. Whether selection for viability changes in direction over time will not be known until the two-year old class of Mixed Spawn is examined.

Selection must also be acting on the genes *Pgm-3* and *Pgi,* or on closely linked loci, to account for their deviations from Hardy-Weinberg expectation. That the situation is complex is shown by the data on joint frequencies. The frequencies of *Lap, Pgm-3* and *Pgi* are clearly not independent of one another and this lack of independence probably reflects their epistatic effect on viability. Mitton *et al.* (1973) have reported such an effect in *Mytilus edulis* between *Lap* and an aminopeptidase locus. In *M. mercenaria,* the departures from independence appear to be specific to particular joint genotypes. No single genotype at one locus interacts strongly with all, or even most, of the genotypes at a second locus. No genotype at one locus shows strong interactions with both other loci. This lack of pattern suggests that entirely different forces are affecting each gene pair.

Of the three loci that have effects on viability, two, *Lap* and *Pgm-3*, also influence shell length. As is the case with the genotype frequency data, the action of closely linked loci cannot be excluded, but we consider it to be unlikely because the association of *Lap* genotypes with size has been recognized in natural populations of other bivalves. Milkman & Koehn (1977) have found that, in *Mytilus edulis, Lap* genotype frequencies vary with size classes because of viability differences rather than effects on growth rates. Zouros *et al.* (1980) have found for oysters, as we do for *Mercenaria*, that genotype does influence growth. The association of genotype with size means that with *M. mercenaria* one must exercise great caution in equating size with age.

The effects that *Lap* and *Pgm-3* have on shell length are real though small (for *Lap,* $R^2 = 0.027$; for *Pgm-3,* $R^2 = 0.010$). In fact, these are polygenes that can be followed as individual Mendelian units. This has allowed us to apply quantitative techniques to estimate the effect of individual alleles. Several studies on the quantitative genetics of oysters (Newkirk *et al.,* 1977; Newkirk, 1978) and mussels (Innes & Haley, 1977) have found both additive and non-additive components to continuous phenotypes. The individually identifiable genes *Lap* and *Pgm-3* in the clam *M. mercenaria* have exactly these effects. The alleles of *Pgm-3* have a straightforward additive effect on shell length while some

alleles of *Lap* interact non-additively. Perhaps many of the known polymorphic loci that code for enzymes will be found to act similarly when the effort is made to associate them with quantitative traits.

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REFERENCES CITED

ADAMKEWICZ, L., TAUB, S. R. & WALL, J. R., 1984, Genetics of the clam *Mercenaria mercenaria*. I. Mendelian inheritance of allozyme variation. *Biochemical Genetics*, 22: in press.

CASTAGNA, M. & KRAEUTER, J., 1981, Manual for growing the hard clam *Mercenaria*. *Special Report in Applied Marine Science and Ocean Engineering* No. 249, Virginia Institute of Marine Science, Gloucester Point, Virginia 23062.

HUMPHREY, C. M., 1982, *Ecological genetics of the hard clams* Mercenaria mercenaria *Linné and* Mercenaria campechiensis *Gmelin: electrophoretic estimation of enzyme variation and the use of shell morphology as a species indicator*. Ph.D. dissertation, University of Georgia.

INNES, D. J. & HALEY, L. E., 1977, Genetic aspects of larval growth under reduced salinity in *Mytilus edulis*. *Biological Bulletin*, 153: 312–321.

KOEHN, R. K., TURANO, F. J. & MITTON, J. B., 1973, Population genetics of marine pelecypods. II. Genetic differences in microhabitats of *Modiolus demissus*. *Evolution*, 27: 100–105.

KOEHN, R. K., MILKMAN, R. & MITTON, J. B., 1976, Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel *Mytilus edulis*. *Evolution*, 30: 2–32.

MILKMAN, R. & KOEHN, R. K., 1977, Temporal variation in the relationship between size, numbers and an allele frequency in a population of *Mytilus edulis*. *Evolution*, 31: 103–115.

MITTON, J. B., KOEHN, R. & PROUT, T., 1973, Population genetics of marine pelecypods. III. Epistasis between functionally related isoenzymes of *Mytilus edulis*. *Genetics*, 73: 487–496.

NEWKIRK, G. F., 1978, Interaction of genotype and salinity in larvae of the oyster *Crassostrea virginica*. *Marine Biology*, 48: 227–234.

NEWKIRK, G. F., HALEY, L. E. & DOYLE, R. W., 1977, Genetics of larvae and spat growth rate in the oyster, *C. virginica*. *Marine Biology*, 41: 49–52.

ZOUROS, E., SINGH, S. M. & MILES, H. E., 1980, Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution*, 34: 856–867.

ZOUROS, E. & FOLTZ, D. W., 1984, Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia*, 25: 583–591.

MALACOLOGIA, 1984, 25(2): 535–549

GENETIC DIVERSITY WITHIN AND BETWEEN POPULATIONS OF AMERICAN OYSTERS (*CRASSOSTREA*)

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ABSTRACT

American oysters of the genus *Crassostrea* form a complex of closely related populations having extremely broad distributions and uncertain systematic affinities. *C. virginica* and *C. rhizophorae* are believed to intergrade in Central America south of the Yucatan Peninsula and on the basis of hybridization studies have been considered subspecies. *C. corteziensis* is said to have diverged in the Pacific Ocean from a *C. virginica* ancestor isolated by the rise of the Central American land bridge.

Electrophoretic studies have been made of a total of 8 populations and 400 individuals to determine the amount of genetic diversity within and between populations and taxa. Averaged over all 8 population samples expected heterozygosity (H_e), proportion of polymorphic loci ($P_{0.95}$), and number of alleles per locus are $H_e = 0.175$, $P_{0.95} = 0.501$, and $n_a = 2.29$, respectively. Excesses of homozygous genotypes over Hardy-Weinberg (random mating) expected proportions within populations are common, but vary among loci and population samples.

Average genetic distance among conspecific populations is less than 0.1, but significant, even diagnostic differences occur at some loci. *C. virginica* from the Bay of Campeche is electrophoretically distinguishable from United States Gulf Coast populations and thus constitutes the fourth race of this species described by such biochemical techniques. *C. rhizophorae* populations in and around the Caribbean show a dispersion of allelic frequencies perhaps indicative of reduced gene flow.

Average genetic distance among species is substantial, about 0.6, indicating that these three taxa are well separated species. Fossil and molecular evidence places the divergence of *C. corteziensis* in the Pliocene after the separation of *C. virginica* from *C. rhizophorae*. The great karyotypic and morphological similarities of these species suggest evolutionary conservation of a basic developmental program.

Key words: American oysters; genetic diversity; molecular *vs.* organismal evolution.

INTRODUCTION

Western Atlantic and Caribbean populations of cupped oysters (*Crassostrea*) comprise perhaps five closely related, morphologically similar, biological species (Ahmed, 1975). Although *C. virginica* is known to include several geographical races, surprisingly little information exists on the population structure and systematic affinities of the other taxa in this economically important species complex. The purposes of this paper are to review the literature on evolutionary divergence in this group of bivalves and to provide biochemical data on genetic diversity within and between its taxa.

C. virginica (Gmelin, 1791), the most widely distributed oyster in this group, ranges from Nova Scotia to the Yucatan Peninsula, Mexico. Geographic variation exists in a variety of physiological processes, activities, tolerances, and in biochemical and serological traits (Hillman, 1964, 1965; Li *et al.*, 1967; Menzel, 1951, 1956; Numachi, 1962; Stauber, 1947, 1950). Yet shell morphology, being heavily influenced by local environmental conditions (Galtsoff, 1964), shows no consistent variation among geographically distant populations. Karyotypes of *C. virginica* from Connecticut (Longwell *et al.*, 1967), northwest Florida (Menzel, 1968a) and Tabasco, Mexico (Rodriguez-Romero *et al.*, 1978) appear to be identical. Gene-enzyme polymorphism as revealed by extensive surveys of electrophoretic variation shows no micro- or macrogeographic variation along the Atlantic seaboard, but Gulf Coast and Canadian populations each have allelic frequencies

markedly different from those in Atlantic populations (W. W. Anderson, University of Georgia, personal communication; Buroker *et al.,* 1979b; N. E. Buroker, Rutgers University, personal communication). Thus, electrophoretic evidence to date indicates that *C. virginica* comprises three races whose ranges exceed the ranges of the physiological races indicated by the earlier literature. No examination of oysters south of the unusual Laguna Madre population (Groue & Lester, 1982) has been made to determine whether the U.S. Gulf Coast race extends to the Yucatan Peninsula.

Divergence of Central and South American populations of *Crassostrea* is not well studied though patterns of species formation in these and other oysters suggest the tropics to be the region of greatest evolutionary activity (Ahmed, 1975). Three *virginica*-like oysters are said to occur in Venezuela, for example (Macsotay, ms; cited by Ahmed, 1975): a local "*C. virginica*," a second differing only by muscle scar pigmentation and size, and a third, the well known mangrove oyster, *C. rhizophorae*, which supposedly replaces *C. virginica* south of Yucatan and is found throughout the Caribbean.

Several workers suggest that *C. rhizophorae* (Guilding, 1828) is either very closely related to, or a variety of, *C. virginica* (Galtsoff, 1964; Korringa, 1952; Mattox, 1949; Menzel, 1968b, 1971; Warmke & Abbott, 1961). Both R. W. Menzel and G. Gunter (personal communication through R.W. Menzel) claim that specimens from the Gulf Coast of Mexico show a gradual transition between *virginica* and *rhizophorae* shell traits such as muscle scar pigmentation and valve shape, articulation and pigmentation. The karyotypes of the two species are practically identical (Menzel, 1968a) although a comparison of chromosome arm ratios in Table 1 of Rodriguez-Romero *et al.* (1978) and Table 1 of Rodriguez-Romero *et al.* (1979a) shows chromosome pair 6 to be metacentric in *C. virginica* and submetacentric in *C. rhizophorae*. The two oysters can be hybridized (Menzel, 1968b, 1971, 1973), and the F_1 larvae show an intergradation between the deeply pigmented valves of *C. rhizophorae* and the lightly colored valves of *C. virginica* (Menzel, personal communication). Because of ease of hybridization, viability of the F_1 through metamoraphosis, and intergradation in F_1 larval shell morphology and pigmentation, Menzel (1973) concludes that *C. rhizophorae* is a subspecies of *C. virginica*. An electrophoretic comparison of *C. rhizophorae* from the Virgin Islands with *C. virginica*, however, reveals substantial biochemical genetic divergence (Buroker *et al.,* 1979b). The systematic affinity of these two oysters thus is in doubt.

Finally, *C. corteziensis* (Hertlein, 1951), which occurs in the Pacific Ocean from Panama to the Gulf of California, differs from *C. virginica* in having either a faint brownish-purple or unpigmented muscle scar (Hertlein, 1951) and a karyotype featuring a metacentric rather than submetacentric chromosome pair 9 and lacking a secondary constriction of the first chromosome pair (Rodriguez-Romero *et al.,* 1979b). Although Hertlein (1951) cites fossil evidence that *C. corteziensis* is of Pliocene age, Stenzel (1971) maintains that this species descended from a panamerican *C. virginica*-like ancestor whose range was divided by the rise of the Central American land bridge.

We report here an electrophoretic survey of gene-enzyme variation within and between population samples of the three species of American oysters. The results bear on the several questions raised by previous work on the systematic affinities of these populations, namely, the degree of divergence of *C. virginica* populations from the U.S. and Mexican Gulf Coasts; the degree of divergence between the readily-hybridized *C. virginica* and *C. rhizophorae* at the gene-enzyme level; and the degree of divergence of *C. corteziensis* from the Atlantic members of the American oyster complex given its minimum geographic isolation of 3 to 4 million years (Keigwin, 1982).

MATERIALS AND METHODS

Samples of American oysters were obtained from the following localities (Fig. 1): (CV1) *C. virginica* from the vicinity of the Florida State University Marine Laboratory, Franklin Co., Florida (N = 99); (CV2) *C. virginica* from Zacatal, Laguna de Terminos, Campeche, Mexico (N = 96); (CR1) *C. rhizophorae* from Belize (N = 45); (CR2) *C. rhizophorae* from Santo Domingo, Dominican Republic (N = 17); (CR3) *C. rhizophorae* from Puerto Rico (N = 43); (CR4) *C. rhizophorae* from Guadeloupe (N = 15); (CR5) *C. rhizophorae* from Trinidad (N = 48); (CC1) *C. corteziensis* from Estero del Pozo near San

Blas, Nayarit, Mexico (N = 23). All specimens were shipped live to the Bodega Marine Laboratory where they were either kept live in a closed, quarantined seawater system or frozen whole at −75°C until dissection for electrophoresis.

Initially, aqueous extracts from homogenized heart, liver, gill, mantle, muscle and gonad tissue samples were subjected to electrophoreses and specific enzyme assays. Because little tissue specificity was detected in the resulting zymograms and because mantle gave slightly better results overall, we performed most electrophoretic comparisons with this tissue. Small oysters were homogenized whole, however. Tissue samples were homogenized in approximately equal volumes of 0.5 M Tris-HCl buffer, pH 7.1, and frozen at −75°C for no more than a few days prior to electrophoresis.

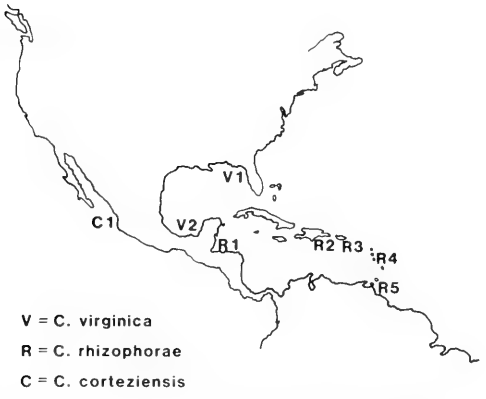

FIG. 1. Collection localities for three species of American oysters (see text for details).

Gel preparation and electrophoretic procedures were substantially those described by Ayala *et al.* (1973) and Tracey *et al.* (1975). Gel-electrode buffer systems A, B and C of Ayala *et al.* (1973) plus an additional system D equivalent to the phosphate-citrate buffer of Selander *et al.* (1971; buffer 8 in their appendix) gave satisfactory resolution of the enzymes listed in Table 1. Enzyme assays are described by Ayala *et al.* (1973), Tracey *et al.* (1975) or references therein, with the exception of aspartate amino transferase (Johnson *et al.*, 1972) and xanthine dehydrogenase (Ayala *et al.*, 1974). No enzymatic activity was detected under our conditions in assays for acid phosphatase, acetaldehyde oxidase, alcohol dehydrogenase, catalase, creatine kinase, fumarase, α-glycerophosphate dehydrogenase, β-hydroxybutyrate dehydrogenase, nucleoside phosphorylase, octanol dehydrogenase, octopine dehydrogenase, and sorbitol dehydrogenase. Assays that gave positive staining but were not resolved well enough or consistently enough to be scored were alkaline phosphatase, esterase, glucose-6-phosphate dehydrogenase, isocitrate dehydrogenase, malic enzyme, mannose-phosphate isomerase, and various peptidases.

Genetic interpretation of zymograms was similar to that of Tracey *et al.* (1975). Putative

TABLE 1. Enzymes surveyed by gel-electrophoresis in the American *Crassostrea* species group, symbols for the loci encoding these enzymes, buffers giving the best resolution (see text), numbers of gene-enzyme systems or loci, and whether the loci are monomorphic (M), polymorphic (P; the 0.95 criterion is met in at least one population sample) or not assayed (NA) (see text).

Enzyme	Genetic symbol	Buffer system	Number of loci	*C. v.*	*C. r.*	*C. c.*
adenylate kinase	*Adk*	C	1	P	P	P
aspartate amino transferase	*Aat*	A	2	2P	1P, 1M	1P, 1M
glutamate dehydrogenase	*Gdh*	B	1	M	M	P
glyceraldehyde-3PO_4-dehydrogenase	*G3pdh*	D	1	M	M	M
hexokinase	*Hk*	B	1	M	P	NA
leucine aminopeptidase	*Lap*	A	2	2P	2P	1NA, 1P
malate dehydrogenase	*Mdh*	C	2	1P, 1M	1P, 1M	1M, 1P
phosphoglucomutase	*Pgm*	D	2	2P	2P	2P
6-phosphogluconate dehydrogenase	*6Pgdh*	C, D*	1	P	P	P
phosphoglucose isomerase	*Pgi*	A	1	P	P	P
protein	*Pt*	A	3	3M	1NA, 2M	3M
triosephosphate isomerase	*Tpi*	B	1	P	P	P
xanthine dehydrogenase	*Xdh*	B	1	P	P	P

*NADP (0.002% w/v) added to gel and electrode buffers.

heterozygotes at the *Aat-1, Aat-2, G3pdh, Mdh, 6Pgdh, Pgi* and *Tpi* loci appeared three-banded indicating dimeric structure for the active enzymes. Monomeric structure was inferred from the two-banded appearance of putative heterozygotes at the *Lap-1, Lap-2,* and *Pgm* loci. Discrimination of *Lap-2* heterozygotes and homozygotes was difficult because of the close spacing of electromorphs. *Pgm* zymograms were complex, comprising the products of at least three loci, and overlap of isozymes may have led to scoring errors for the *Pgm* loci. The band structures of heterozygotes at the remaining polymorphic loci were not well enough resolved to suggest apparent subunit compositions of the active enzymes.

Multiple loci encoding the same enzyme are designated by numerical suffices of the gene symbol with "−1" denoting the most slowly migrating isozyme. Alleles at each locus are designated by adding or subtracting the number of millimeters by which migration of the corresponding electromorph differs from that of the protein encoded by a standard allele arbitrarily named "100." The standard alleles at each locus are the most frequent alleles encountered in the Florida *C. virginica* population sample.

Raw data consisting of single-individual, multilocus genotypes were subjected to the Biosys-1 Fortran computer program of Swofford & Selander (1981) for calculations of: allelic frequencies in each population; goodness-of-fit to Hardy Weinberg (H-W) expected genotypic proportions; average observed and expected proportions of heterozygotes per locus (H_o and Nei's, 1978, unbiased estimates of H_e); proportions of polymorphic loci per population, using stringent and less stringent criteria of polymorphism, *i.e.* the frequency of the most common allele does not exceed 0.95 ($P_{0.95}$) or 0.99 ($P_{0.99}$), respectively; mean numbers of alleles per locus (n_a); Wright's (1943) hierarchical *F* statistics; Nei's (1978) unbiased measures of genetic similarity (*I*) and distance (*D*); Cavalli-Sforza & Edwards' (1967) arc distances; and a Wagner network based on the arc distances. Because not all loci were scored in each population sample, genetic distance analyses were restricted to the CV1, CR1, CR2, CR5, and CC1 samples for which the same set of 15 gene-enzyme systems could be compared (see Table 2). Averaging of *H* or *P* statistics over groups of population samples for purposes of testing differences in means was done using the angular transformation, $\sin^{-1}\sqrt{x}$.

RESULTS

Variation within populations

Population samples of American oysters are genetically quite variable. Frequencies of all electromorphs detected in a survey of 19 enzymes and proteins are given in Table 2. An obvious problem with this data set is missing information for various enzymes in all but the CV1 sample. Because genetic variation is very unevenly distributed across loci, such missing data make comparisons among populations difficult. For this reason summary statistics are presented (Table 3) not only for the eight original population samples but also for those five population samples that may be compared at the same set of 15 loci. These comparable samples are designated by asterisks following the population sample codes. Thus, CV1 was surveyed at all 19 proteins, but the CV1* data set is based only upon the 15 loci surveyed in the other four asterisked samples.

The two population samples of *C. virginica* are the best studied in terms of numbers of individuals assayed and, for the Florida sample at least, the number of loci surveyed. The Campeche sample appears to have slightly more variation than the Florida sample (n_a = 2.94 vs. 2.42; $P_{0.95}$ = 56.3% vs. 47.4%; H_e = 26.5% vs. 20.6%). This cannot be attributed to a bias in the loci sampled since the three loci not scored in the Mexican sample are polymorphic systems in all other population samples. Moreover, locus by locus paired comparisons show that heterozygosities of the Campeche sample are greater than those of the Florida sample at all loci but *Tpi*; the average difference in paired, transformed expected heterozygosities is significantly different than zero (t = 2.485, 10 df., $0.02 < p < 0.05$).

Five *C. rhizophorae* population samples appear to be slightly less variable than the *C. virginica* samples although fewer individuals and loci have been assayed. The mean number of alleles per locus ranges from 1.73 to 2.60 with the lowest values corresponding to the samples having the smallest numbers of individuals, CR2 and CR4. Proportions of polymorphic loci per population, on the other hand, are quite comparable to those in *C. virginica*; CR3's low $P_{0.95}$ of 36.4% is the

TABLE 2. Electromorph frequencies at 19 gene-enzyme systems studied in eight populations of American oysters (*Crassostrea*). See text for locality information. (N) is the number of individuals assayed.

		C. virginica		*C. rhizophorae*					*C. cortez.*
Locus	Alleles	CV1	CV2	CR1	CR2	CR3	CR4	CR5	CC1
	(N) =	47	—	43	17	37	—	47	22
	95	0.0	—	0.0	0.0	0.068	—	0.0	0.0
	96	0.011	—	0.0	0.0	0.095	—	0.0	0.0
	98	0.096	—	0.0	0.0	0.838	—	0.0	0.023
	100	0.447	—	0.035	0.0	0.0	—	0.0	0.841
Adk	101	0.0	—	0.0	0.0	0.0	—	0.011	0.0
	102	0.447	—	0.0	0.0	0.0	—	0.0	0.136
	103	0.0	—	0.071	0.441	0.0	—	0.979	0.0
	106	0.0	—	0.860	0.559	0.0	—	0.011	0.0
	108	0.0	—	0.035	0.0	0.0	—	0.0	0.0
	(N) =	95	72	45	16	47	15	24	23
	86	0.005	0.014	0.0	0.0	0.0	0.0	0.0	0.0
	92	0.537	0.528	0.0	0.031	0.0	0.0	0.0	0.652
	95	0.0	0.0	0.0	0.0	0.0	0.067	0.0	0.0
Aat-1	100	0.458	0.451	0.978	0.875	1.000	0.933	1.000	0.348
	104	0.0	0.0	0.011	0.094	0.0	0.0	0.0	0.0
	106	0.0	0.007	0.0	0.0	0.0	0.0	0.0	0.0
	110	0.0	0.0	0.011	0.0	0.0	0.0	0.0	0.0
	(N) =	75	72	22	4	19	13	8	22
	96	0.0	0.493	0.0	0.0	0.0	0.0	0.0	0.0
Aat-2	100	1.000	0.0	1.000	1.000	1.000	1.000	1.000	1.000
	102	0.0	0.507	0.0	0.0	0.0	0.0	0.0	0.0
	(N) =	95	96	26	15	12	15	21	23
Gdh	100	1.000	1.000	0.0	0.0	0.0	0.0	0.0	0.043
	101	0.0	0.0	1.000	1.000	1.000	1.000	1.000	0.957
	(N) =	95	50	44	17	43	15	48	23
	97	0.0	0.0	1.000	1.000	1.000	1.000	1.000	0.0
	98	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000
G3pdh	100	0.995	0.980	0.0	0.0	0.0	0.0	0.0	0.0
	105	0.005	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	106	0.0	0.020	0.0	0.0	0.0	0.0	0.0	0.0
	(N) =	95	79	—	—	—	15	47	—
Hk	97	0.0	0.0	—	—	—	0.0	0.117	—
	100	1.000	1.000	—	—	—	1.000	0.883	—
	(N) =	96	58	—	—	—	—	48	—
	94	0.021	0.034	—	—	—	—	0.0	—
	95	0.010	0.0	—	—	—	—	0.0	—
	97	0.151	0.164	—	—	—	—	0.0	—
	98	0.089	0.009	—	—	—	—	0.0	—
Lap-1	100	0.667	0.440	—	—	—	—	0.010	—
	102	0.063	0.017	—	—	—	—	0.0	—
	103	0.0	0.336	—	—	—	—	0.0	—
	104	0.0	0.0	—	—	—	—	0.865	—
	106	0.0	0.0	—	—	—	—	0.125	—
	(N) =	73	81	39	27	43	15	44	16
	97	0.0	0.025	0.0	0.0	0.0	0.0	0.091	0.313
	98	0.219	0.043	0.372	0.441	0.070	0.533	0.284	0.0
	99	0.0	0.198	0.0	0.0	0.0	0.0	0.0	0.0
Lap-2	100	0.781	0.494	0.615	0.559	0.894	0.467	0.500	0.531

TABLE 2. (Continued)

		C. virginica		*C. rhizophorae*					*C. cortez.*
Locus	Alleles	CV1	CV2	CR1	CR2	CR3	CR4	CR5	CC1
Lap-2	101	0.0	0.235	0.0	0.0	0.0	0.0	0.0	0.0
	102	0.0	0.006	0.013	0.0	0.081	0.0	0.125	0.0
	103	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.125
	104	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	105	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.031
	(N) =	99	52	45	17	47	—	38	23
	92	0.005	0.0	0.0	0.0	0.0	—	0.0	0.0
	96	0.005	0.317	0.011	0.0	0.0	—	0.0	0.0
	98	0.0	0.0	0.0	0.0	0.011	—	0.0	0.0
Mdh-1	100	0.955	0.500	0.0	0.0	0.0	—	0.0	1.000
	102	0.0	0.0	0.911	0.941	0.947	—	0.921	0.0
	105	0.035	0.183	0.0	0.0	0.0	—	0.0	0.0
	108	0.0	0.0	0.078	0.059	0.043	—	0.079	0.0
	(N) =	99	96	45	17	47	14	47	23
	94	0.0	0.0	0.978	1.000	1.000	0.964	1.000	0.935
Mdh-2	99	0.0	0.0	0.022	0.0	0.0	0.036	0.0	0.065
	100	1.000	0.995	0.0	0.0	0.0	0.0	0.0	0.0
	104	0.0	0.005	0.0	0.0	0.0	0.0	0.0	0.0
	(N) =	28	—	43	17	43	13	42	22
	90	0.0	—	0.0	0.0	0.0	0.0	0.012	0.0
	94	0.0	—	0.023	0.0	0.0	0.0	0.083	0.0
	98	0.107	—	0.012	0.0	0.116	0.038	0.131	0.0
Pgm-1	100	0.839	—	0.779	0.794	0.756	0.885	0.667	0.886
	102	0.054	—	0.186	0.118	0.105	0.0	0.071	0.114
	104	0.0	—	0.0	0.088	0.0	0.0	0.024	0.0
	105	0.0	—	0.0	0.0	0.012	0.077	0.0	0.0
	107	0.0	—	0.0	0.0	0.012	0.0	0.012	0.0
	(N) =	11	—	41	17	—	15	11	21
	96	0.0	—	0.0	0.0	—	0.0	0.0	0.0
	100	0.636	—	0.061	0.0	—	0.0	0.0	0.0
	102	0.364	—	0.0	0.0	—	0.0	0.0	0.762
	104	0.0	—	0.012	0.0	—	0.0	0.091	0.0
Pgm-2	106	0.0	—	0.744	0.912	—	0.900	0.818	0.238
	108	0.0	—	0.0	0.0	—	0.0	0.0	0.0
	109	0.0	—	0.183	0.088	—	0.100	0.091	0.0
	110	0.0	—	0.0	0.0	—	0.0	0.0	0.0
	(N) =	86	78	—	—	—	11	48	23
	94	0.0	0.0	—	—	—	0.0	0.042	0.0
	96	0.070	0.109	—	—	—	0.955	0.875	0.109
	99	0.0	0.0	—	—	—	0.0	0.073	0.0
6Pgdh	100	0.907	0.859	—	—	—	0.0	0.0	0.848
	101	0.0	0.0	—	—	—	0.045	0.010	0.0
	103	0.017	0.013	—	—	—	0.0	0.0	0.0
	104	0.006	0.019	—	—	—	0.0	0.0	0.022
	107	0.0	0.0	—	—	—	0.0	0.0	0.022
	(N) =	99	91	45	17	47	15	48	23
	90	0.010	0.016	0.0	0.0	0.0	0.0	0.0	0.174
	91	0.0	0.0	0.0	0.0	0.0	0.0	0.010	0.0
	94	0.0	0.005	0.0	0.0	0.0	0.0	0.0	0.0
	95	0.263	0.484	0.011	0.0	0.0	0.033	0.063	0.022
Pgi	97	0.0	0.0	0.011	0.0	0.0	0.0	0.0	0.0

TABLE 2. (Continued)

		C. virginica		*C. rhizophorae*					*C. cortez*
Locus	Alleles	CV1	CV2	CR1	CR2	CR3	CR4	CR5	CC1
Pgi	100	0.621	0.445	0.844	0.853	0.979	0.867	0.823	0.739
	102	0.0	0.005	0.011	0.0	0.0	0.0	0.0	0.0
	103	0.0	0.0	0.0	0.0	0.021	0.0	0.0	0.0
	104	0.106	0.044	0.100	0.147	0.0	0.033	0.104	0.065
	107	0.0	0.0	0.022	0.0	0.0	0.067	0.0	0.0
	(N) =	58	39	—	—	—	—	—	6
Pt-1	100	1.000	1.000	—	—	—	—	—	1.000
	(N) =	55	14	8	8	—	4	8	8
Pt-2	100	1.000	1.000	1.000	1.000	—	1.000	1.000	1.000
	(N) =	72	29	28	16	—	13	8	12
Pt-3	100	1.000	1.000	1.000	1.000	—	1.000	1.000	1.000
	(N) =	77	76	44	15	—	7	47	23
	85	0.0	0.0	0.023	0.600	—	0.0	0.138	0.0
	90	0.0	0.0	0.0	0.0	—	0.0	0.0	0.065
	93	0.169	0.039	0.909	0.367	—	0.857	0.840	0.0
	95	0.0	0.0	0.0	0.0	—	0.0	0.0	0.848
Tpi	96	0.0	0.013	0.0	0.0	—	0.0	0.0	0.0
	98	0.0	0.0	0.0	0.0	—	0.143	0.021	0.0
	100	0.792	0.914	0.0	0.033	—	0.0	0.0	0.087
	101	0.0	0.0	0.068	0.0	—	0.0	0.0	0.0
	105	0.039	0.007	0.0	0.0	—	0.0	0.0	0.0
	106	0.0	0.026	0.0	0.0	—	0.0	0.0	0.0
	(N) =	93	76	43	17	47	15	12	22
	95	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.886
	97	0.011	0.132	0.012	0.0	0.021	0.0	0.0	0.0
	99	0.0	0.0	0.0	0.0	0.0	0.0	0.042	0.114
Xdh	100	0.989	0.868	0.988	1.000	0.968	0.900	0.625	0.0
	102	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	104	0.0	0.0	0.0	0.0	0.011	0.100	0.0	0.0
	105	0.0	0.0	0.0	0.0	0.0	0.0	0.333	0.0

result of missing data at several loci that are moderately polymorphic in the other *C. rhizophorae* population samples. Expected heterozygosity, perhaps the best measure of gene diversity (Nei & Roychoudhury, 1974), is consistently but not significantly lower in *C. rhizophorae* than in *C. virginica*. A *t*-test of the significance of the difference between the CV1* transformed H_e value and the mean of the CR1*, CR2* and CR5* transformed H_e values yields $t = 1.852$, 2 df., $0.10 < p < 0.15$; the mean of paired locus differences does not differ from zero in any of the three comparisons.

Finally, the single *C. corteziensis* sample is intermediate to the other American oyster population samples in measures of genetic diversity.

In all eight population samples there is a consistent trend toward deficiency of the average proportion of heterozygous genotypes per locus with respect to random mating proportions (Table 3). The proportional deviation (D = (obs. − exp.)/exp.; Selander, 1970) calculated from the observed and expected numbers of heterozygous genotypes summed over all loci ranges from −33.5% in the CC1* sample to −0.3% in the CV2 sample. A paired comparison of the eight transformed mean H_o and H_e shows this trend to be significant ($t = 3.67$, 9 df., $p < 0.01$) although within certain populations, such as the *C. virginica* samples, the overall agreement with H-W genotypic proportions appears to be quite good.

TABLE 3. Summary statistics of genetic variation in eight population samples of American oysters (*Crassostrea*). Localities are defined in the text; data in boldface for * localities are based on the same set of 15 loci. L, number of loci studied; N, number of individuals assayed; n_a, mean number of alleles detected per locus; P_{99} and P_{95}, percentages of loci polymorphic per population using less stringent and stringent definitions of polymorphism, respectively (see text); H_o and H_e, mean percentages of heterozygotes per locus observed and expected (unbiased estimate of Nei, 1978), respectively; D, difference between the observed and expected numbers of heterozygotes summed over loci as a percentage of the expected number.

	C. virginica			*C. rhizophorae*						*C. corteziensis*	
Statistic	CV1	CV1 *	CV2	CR1 *	CR2 *	CR3	CR4	CR5	CR5 *	CC1	CC1 *
L	19	**15**	16	**15**	**15**	11	15	18	**15**	17	**15**
N	76.2	**74.2**	66.2	**37.4**	**15.1**	39.6	13.0	33.1	**30.2**	19.7	**20.4**
(S.E.)	(5.9)	**(7.2)**	(6.0)	**(2.9)**	**(1.0)**	(3.7)	(0.9)	(4.0)	**(4.5)**	(1.4)	**(1.2)**
n_a	2.42	**2.27**	2.94	**2.60**	**1.73**	2.18	1.80	2.50	**2.40**	2.12	**2.07**
(S.E.)	(0.34)	**(0.30)**	(0.49)	**(0.39)**	**(0.21)**	(0.40)	(0.22)	(0.38)	**(0.45)**	(10.27)	**(0.27)**
P_{99}	57.9	**60.0**	62.5	**66.7**	**53.3**	54.6	60.0	61.1	**53.3**	64.7	**66.7**
P_{95}	47.4	**46.7**	56.3	**46.7**	**53.3**	36.4	46.7	55.6	**46.7**	58.8	**66.7**
H_o	17.6	**18.1**	26.3	**13.6**	**13.8**	10.6	9.9	17.0	**16.2**	13.8	**13.6**
(S.E.)	(4.5)	**(5.2)**	(7.0)	**(3.8)**	**(4.1)**	(4.3)	(2.7)	(4.1)	**(4.9)**	(3.4)	**(3.6)**
H_e	20.6	**21.5**	26.5	**15.0**	**17.8**	10.7	12.7	19.4	**18.7**	19.6	**20.4**
(S.E.)	(5.3)	**(6.1)**	(6.9)	**(4.4)**	**(5.4)**	(4.4)	(3.8)	(5.0)	**(6.0)**	(4.7)	**(5.2)**
D	−9.2	**−8.7**	−0.3	**−8.5**	**−22.8**	−1.3	−26.0	−13.0	**−15.0**	−29.6	**−33.5**

Genetic distance between conspecific populations

Florida and Campeche population samples of *C. virginica* have significantly different allelic frequencies at several polymorphic loci (*Lap-1, Lap-2, Mdh-1, Pgi, Tpi* and *Xdh*), and share no alleles at the *Aat-2* locus (Table 2). The average fixation index for individuals within these subpopulations, $F_{IS} = 0.015$, indicates little deviation from random mating proportions as noted above. By contrast the standardized variance of allele frequencies between these populations, $F_{ST} = 0.149$, indicates substantial divergence between Florida Gulf Coast and Gulf of Campeche *C. virginica*. Average genetic similarity and distance over the 16 loci that may be compared in these two population samples are $I = 0.910$ and $D = 0.094$ (included in Table 5 though not strictly comparable to other values given there).

A similar level of divergence is evident among conspecific populations of *C. rhizophorae*. Comparing just the CR1*, CR2* and CR5* samples, for example, there is significant heterogeneity of allelic frequencies at the *Aat-1, Adk, Lap-2, Pgm-1, Tpi* and *Xdh* loci, and over all loci $F_{ST} = 0.197$. Average genetic similarity and distance among the CR1*, CR2* and CR5* are $I = 0.947$ and $D = 0.055$ (Tables 4 and 5). Examination of allelic frequencies for the CR3 and CR4 samples (Table 2) reveals similar levels of divergence from the other conspecific population samples; in the six pairwise comparisons between these two samples and the other three populations, average $I = 0.947$ (11–15 loci) and average $F_{ST} = 0.140$. The distribution of single-locus genetic identity values in 61 comparisons between conspecific populations (Fig. 2) illustrates the overall high level of similarity with 80% of the comparisons yielding $I > 0.95$. The occurrence of loci in the middle and lower end of this distribution, however, indicates that substantial or complete divergence of allelic frequencies has occurred among geographically distant conspecific populations of American oysters.

Genetic distance between American oyster species

Genetic divergence among the species of the American *Crassostrea* group is considerably greater than that observed in com-

TABLE 4. Genetic similarity (Nei's, 1978, unbiased identity above diagonal) and genetic distance (Nei's 1978 unbiased statistic below diagonal) averaged over 15 gene-enzyme systems studied in five populations of American oysters (*Crassostrea*). Conspecific comparisons indicated by italics.

	CV1*	CR1*	CR2*	CR5*	CC1*
C. virginica					
CV1* Florida	—	0.522	0.518	0.483	0.584
C. rhizophorae					
CR1* Belize	0.650	—	*0.966*	*0.926*	0.575
CR2* Santo Domingo	0.657	*0.034*	—	*0.947*	0.584
CR5* Trinidad	0.728	*0.077*	*0.054*	—	0.576
C. corteziensis					
CC1* San Blas	0.537	0.554	0.537	0.552	—

TABLE 5. Average genetic similarity (above diagonal) and distance (below diagonal) for intra- and interspecific comparisons among five populations of three American oysters (*Crassostrea*) assayed for variation at 15 gene-enzyme systems.

Species	No. of pops.	1	2	3
1. *C. virginica*	1	(0.094–0.910)*	0.508	0.584
2. *C. rhizophorae*	3	0.678	0.055–0.947	0.578
3. *C. corteziensis*	1	0.537	0.548	—

*Similarity and distance statistics for a comparison of Florida and Campeche *C. virginica* populations are based on a slightly different set of 16 loci (see Table 2), but are presented here for comparison to the conspecific *C. rhizophorae* statistics.

parisons of conspecific population samples. Genetic identity and distance between Florida *C. virginica* and the three *C. rhizophorae* samples assayed for the same set of 15 gene-enzyme systems range from $I = 0.483$ to 0.522 (Table 4) with an average of $I = 0.508$ (Table 5) and from $D = 0.650$ to 0.728 (Table 4) with an average of $D = 0.678$ (Table 5). Genetic divergence between *C. virginica* and *C. corteziensis* is only about 80% of that in the *virginica–rhizophorae* comparison ($I = 0.584$ and $D = 0.537$, Table 5), and about the same as the divergence between *C. rhizophorae* and *C. corteziensis* ($I = 0.578$ and $D = 0.548$, Table 5). The distribution of single-locus genetic identity values in comparisons between population samples of the three species (Fig. 3) is dramatically different from the distribution shown below for conspecific comparisons (Fig. 2). Where only one conspecific comparison (*Aat-2* between CV1 and CV2) lies in the identity interval of 0.0 to 0.05, 34% of the 105 species comparisons are found in this identity class; conversely, where 80% of conspecific comparisons fall in the interval of highest identity (0.95 to 1.0), only 41% of specific comparisons remain.

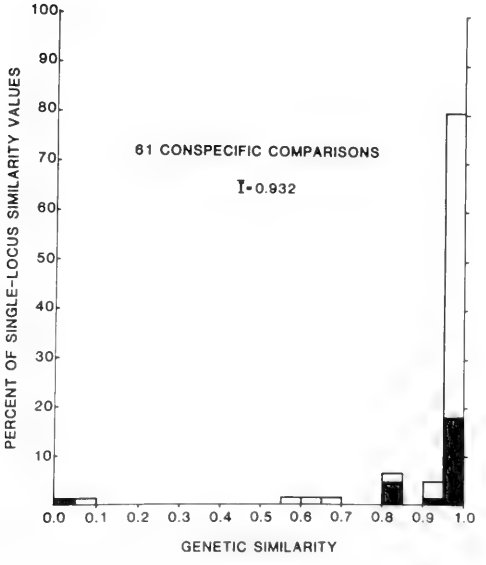

FIG. 2. Distribution of single-locus identity values in comparisons among conspecific American oyster populations. Black bars indicate the distribution of values from the comparison of two *C. virginica* samples since these are based on a slightly different set of 16 loci than the 15 loci assayed in comparisons among three *C. rhizophorae* samples.

Cladistic relationships among American oyster populations

For purposes of constructing a phylogenetic tree using the distance Wagner procedure (a routine available on the Biosys-1 computer program of Swofford & Selander, 1981), Cavalli-Sforza & Edwards' (1967) arc distances were computed since Nei's distance statistic is nonmetric. Addition of taxonomic units was determined by the third criterion of Swofford (1981), and the tree was rooted at the midpoint of the path of greatest patristic difference (CV1* to CR5*). After op-

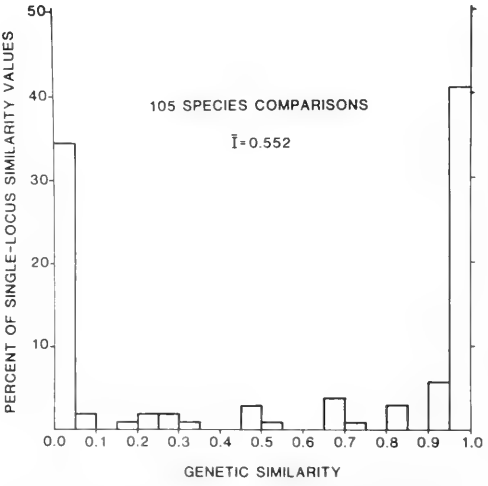

FIG. 3. Distribution of single-locus identity values at 15 loci in comparisons among five populations from three species of American oysters.

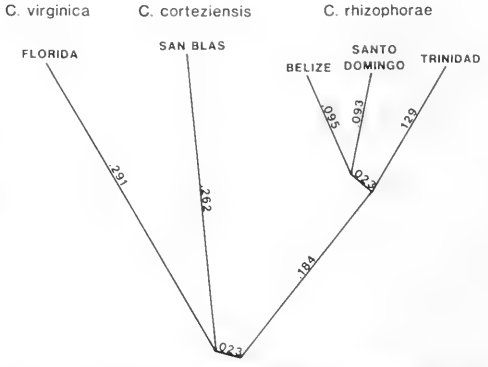

FIG. 4. Distance Wagner network representing the phylogenetic relationships of five American oyster populations. Numbers on branches are Cavalli-Sforza & Edwards' (1967) arc distance values.

timization of branch lengths, the tree (Fig. 4) yielded a cophenetic correlation coefficient (Sneath & Sokal, 1973) of 0.999.

DISCUSSION

Like other bivalves that have been surveyed for genic variation by electrophoretic techniques, the three species of American oysters we have studied are very polymorphic. Over all eight population samples grand averages for three measures of genic variability are: n_a = 2.29; $P_{0.99}$ = 60.1% and $P_{0.95}$ = 50.1%; H_o = 15.0% and H_e = 17.5%.

C. rhizophorae appears to have less variation than *C. virginica* (for CV1* versus the average of CR1*, CR2*, and CR3* mean H_e = 21.5 vs. 17.1, respectively) although the difference is not significant due to large errors in estimates of this quantity. Nevertheless, such a difference is also reported by Buroker *et al.* (1979b) for a west Florida sample of *C. virginica* and a Virgin Island sample of *C. rhizophorae*. These authors hypothesize that variability in Caribbean island populations of *C. rhizophorae* may have been reduced as the result of colonization by relatively small founder populations. Our results are equivocal on this point. The mainland Belize sample has a level of enzyme polymorphism that is intermediate with respect to the island *C. rhizophorae* samples. Belize, however, is near the northern mainland limit of the mangrove oyster, and the level of variation in its native populations may be reduced compared to mainland populations from the center of the species' range. The most variable population sample, from Trinidad, an island that is essentially part of the Venezuelan coast (Darlington, 1957), may attest to higher levels of variation in central, mainland *C. rhizophorae* populations. More comparisons of mainland and island populations will be needed to test adequately this hypothesis.

Many electrophoretic studies of bivalves have revealed deficiencies in proportions of heterozygous genotypes with respect to proportions expected under H-W random-mating equilibrium (see Singh & Green, 1984; Zouros & Foltz, 1984). This study proves no exception with observed heterozygosity less than expected in every population sample, the average difference between the two measures being significantly different than zero. At least a portion of this deficiency, however, may be due to biases in scoring *Lap* and *Pgm* phenotypes (see Materials and Methods). Deficiencies of heterozygotes at the *Lap-2* locus occur in all populations and are significant in the CR2 and CR4 samples. Heterozygote deficiency at *Pgm-1* occurs in five of seven samples and is significant in the CR2 sample.

On the other hand departures from random mating proportions are found at loci whose phenotypes are clear, well separated and unambiguously scored, such as *Mdh-2*, *Pgi*, *6Pgdh* and *Tpi*. Excess homozygosity in the *C. corteziensis* sample is particularly striking at the following loci: *Gdh* (one homozygote for the *100* allele with no heterozygotes), *Pgi* (three homozygotes for the rare *90* allele and only two heterozygotes with the common *100* allele) and *Tpi* (the *100* allele occurs only in two homogyzotes). These homozygotes are distributed over different individuals so that the joint homozygote excess at these three loci cannot be due to the inclusion of individuals from a cryptic species having different gene frequencies. Thus, although our population samples are too small to permit us to estimate homozygote excess with any precision, we do conclude that distributions of genotypes in most American oyster populations depart from H-W expected distributions.

Significant, and even diagnostic, gene-enzyme differences occur between the two samples of *C. virginica* and among the five samples of *C. rhizophorae*. Divergence of Campeche *C. virginica* from conspecific populations along the U.S. Gulf Coast is complete at the *Aat-2* locus—the Campeche sample is polymorphic for alleles *96* and *102* while the Florida sample is fixed for the *100* allele—and is statistically significant at six other polymorphic loci. Average genetic distance over 16 loci in the comparison of CV1 and CV2 is D = 0.094 which is only about half as large as the distance between west Florida and Nova Scotia *C. virginica* reported by Buroker *et al.* (1979b). Nevertheless, this level of divergence is substantially greater than the D = 0.01 observed among contiguous, conspecific populations of *C. gigas, Saccostrea commercialis* (Buroker *et al.,* 1979a) and U.S. Atlantic or U.S. Gulf Coast *C. virginica* (W. W. Anderson and N. E. Buroker, personal communications). Moreover, the presence of one fixed difference and significant differences in several allele-frequency profiles suggest lack of effective gene flow between U.S. Gulf Coast and Bay of Campeche oyster populations.

Our results apparently raise the number of racially distinct populations of *C. virginica* that have been detected by electrophoretic methods to four—the Canadian, U.S. Atlantic Coast, U.S. Gulf Coast, and Bay of Campeche populations. The relationship between the unusual Laguna Madre, Texas population (Groue & Lester, 1982) and the Bay of Campeche population remains to be established. Much more sampling is needed in regions where these natural populations are likely to come into contact or to be separated by geographic or habitat barriers in order to elucidate the taxonomic status and evolutionary relationships of these races of American oysters.

The samples of *C. rhizophorae* examined are quite heterogeneous with respect to allelic frequencies; standardized variance of allelic frequency averaged over all alleles and all loci in the CR1*, CR2* and CR5* samples is F_{ST} = 0.197, slightly higher than the value of 0.149 calculated for the *C. virginica* samples. With the exception of *Adk* in the Puerto Rico sample, however, geographic variation in the mangrove oyster does not comprise diagnostic differences; rather, divergence among populations is due to unpatterned dispersion of gene frequencies at many loci (Table 2). There is also a high proportion of rare alleles that occur in only one population sample; of 58 alleles at 15 loci assayed in at least four of the five *C. rhizophorae* populations, 21 (36%) are unique to one sample.

Divergence of *C. rhizophorae* populations might be due to founder effects associated with colonization of the Caribbean islands, but as noted above, the present evidence on levels of polymorphism in mainland versus island populations does not strongly support this hypothesis. Even if mainland and island populations have similar effective sizes, random genetic drift might be responsible for the observed dispersion of allele frequencies provided that gene flow among these populations has been restricted for a sufficient period. This is a possibility given the apparent isolation of the West Indian and Caribbean Faunal Provinces (Briggs, 1974). Finally, diversifying natural selection may be responsible for divergence of these populations, but we have no evidence for or against this mechanism. It seems likely that gene flow is restricted at least among certain of these populations, and more studies of how recruitment occurs are needed to clarify the population structure of *C. rhizophorae*.

By comparison to the genetic divergence among conspecific populations, the genetic distance among the species of American oysters is large indeed. *C. virginica* and *C. rhizophorae* are the most divergent with estimated average genetic distance of D = 0.678 and similarity of I = 0.508. This divergence is twice as large as that reported by Buroker *et al.* (1979b), D = 0.325 and I = 0.722, in their comparison of west Florida *C. virginica* and Virgin Island *C. rhizophorae*. As their sample of *C. virginica* came from nearly the same locality as ours this difference in results is problematical.

Comparing their Table 1 to our Table 2, we find only ten loci in common that underlie our respective estimates of genetic distance. In this set of common loci, agreement is quite good at all but the two *Mdh* loci which they find both identical in the two species and which we find both fixed for different alleles. Buroker *et al.* (1979b) sampled an additional 20 loci which we have not sampled and found 14 with high similarity and 6 with low similarity between the species; taking high similarity to be 1.0 and low similarity to be 0.0 (effectively the situation as illustrated in Fig. 3) the average similarity in this group of loci, 0.7, is very close to their overall average of 0.72. Of the five loci we have assayed which Buroker *et al.* (1979b) did not study, we find three with high and two with low similarity for an average of 0.6, above our overall average of 0.51. Thus, we have probably underestimated the similarity in our small sample of loci, while Buroker *et al.* (1976b) probably overestimated similarity by not resolving differences at the two *Mdh* loci.

A surprising result is the intermediate position of *C. corteziensis* with respect to *C. virginica* and *C. rhizophorae* (D = 0.537 and D = 0.548, respectively, compared to D = 0.678 for the *virginica-rhizophorae* comparison. The phylogenetic tree drawn from the arc distance matrix for the CV1*, CR1*, CR2*, CR5* and CC1* population samples (Fig. 4) implies that the divergence of the Atlantic and Caribbean species preceded the evolution of the Pacific species. Ages of the *corteziensis-virginica* and *corteziensis-rhizophorae* separations relative to the *virginica-rhizophorae* split are estimated as the ratios of the appropriate D values, or 0.537 / 0.678 = 0.79 and 0.548 / 0.678 = 0.81, respectively (Nei, 1971).

Many authors have suggested that divergence in the amino acid sequences of

proteins is proportional to time since evolutionary separation of taxa (the molecular clock hypothesis reviewed by Wilson *et al.*, 1977). The clock has been calibrated in disparate ways, however, yielding quite disparate estimates of divergence times. For the separation of *C. virginica* from *C. rhizophorae*, for example, estimates of evolutionary age are 1.4×10^6, 3.4×10^6 and 12.8×10^6 years by the methods of Carson (1976), Nei (1975) and Carlson *et al.* (1978), respectively. The smallest estimate of evolutionary age appears to be an underestimate since it is less than the age of the Central American land barrier (3–4 million years; see Keigwin, 1982). The middle estimate is compatible with the age of this land barrier while the largest estimate suggests that the three species of American oysters diverged from one another during the late Miocene or early Pliocene when the Lesser Antillean island chain was emerging and much before the rise of the Isthmus (see Yang *et al.*, 1974). Indeed, this last phylogenetic scenario is compatible with fossil evidence that *C. corteziensis* dates back to the Pliocene (Hertlein, 1951).

Lessios (1979, 1981) has argued against the molecular clock hypothesis on the basis of heterogeneity of genetic distance estimates for comparisons of Pacific and Caribbean geminate species of sea urchins. The distances for species pairs of *Diadema, Eucidaris* and *Echinometra* are 0.026, 0.329 and 0.593 (average of two comparisons), respectively. Vawter *et al.* (1980) have criticized Lessios (1979) for relying on a small number of comparisons in rejecting the clock hypothesis, and have themselves argued that an average distance of 0.214 (range: 0.131 to 0.361) over ten interoceanic comparisons of fish populations supports the molecular clock hypothesis. Vawter *et al.* (1980) also point out that large distances such as observed by Lessios between *Echinometra* species (and by us between the supposedly geminate *Crassostrea* species) cannot be used to test the molecular clock hypothesis since their divergence may have antedated the rise of the Central American land barrier. Given the weight of evidence that proteins do diverge as a function of time, that the magnitude of divergence among American oysters is large with respect to the average of all transisthmian comparisons made to date, and finally, that shells of *C. corteziensis* have been identified in Pliocene beds, we conclude that American oysters evolved much before the final geographic isolation of *C. corteziensis* from the Atlantic members of the species group.

Despite their chromosomal and morphological similarity and their ability to produce viable F_1 hybrids in artificial crosses (Menzel 1968b, 1971, 1973; Rodriguez-Romero *et al.*, 1979a, 1979b), *C. virginica* and *C. rhizophorae* have clearly evolved protein differences characteristic of old and well-separated biological species. Disparity in rates of evolution at different levels of phenotypic organization, however, has long been recognized. As Dobzhansky (1970) notes, "This is what Harland (1936) meant by his dictum, 'The modifiers really constitute the species.'" Recently, Wilson (1976) and colleagues have called attention to discrepancies in rates of molecular vs. organismal evolution in different groups of organisms. Frogs, relative to mammals for example, are an old lineage that has undergone relatively minor morphological evolution (Cherry *et al.*, 1978), little chromosomal or karyotypic reorganization (Wilson *et al.*, 1974b; Wilson *et al.*, 1975), a lineage that evidences broad tolerance and often a proclivity for interspecific hybridization (Wilson *et al.*, 1974a), yet is generally characterized by large species differences at the molecular level. The similarity of American oysters to frogs in this respect suggests that a basic developmental, gene-regulatory program has been simply conserved during the evolution of this complex of oyster species.

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LITERATURE CITED

AHMED, M., 1975, Speciation in living oysters. *Advances in Marine Biology,* 13: 375–397.

AYALA, F. J., HEDGECOCK, D., ZUMWALT, G. S. & VALENTINE, J. W., 1973, Genetic variation in *Tridacna maxima,* an ecological analog of some unsuccessful evolutionary lineages. *Evolution,* 27: 171–191.

AYALA, F. J., TRACEY, M. L., BARR, L. G. & EHRENFELD, J. G., 1974, Genetic and reproductive differentiation of the subspecies, *Drosophila equinoxialis caribbensis. Evolution,* 28: 24–41.

BRIGGS, J. C., 1974, *Marine zoogeography.* McGraw-Hill, New York, 475 p.

BUROKER, N. E., HERSHBERGER, W. K. & CHEW, K. K., 1979a, Population genetics of the family Ostreidae. I. Intraspecific studies of *Crassostrea gigas* and *Saccostrea commercialis. Marine Biology,* 54: 157–169.

BUROKER, N. E., HERSHBERGER, W. K. & CHEW, K. K., 1979b, Population genetics of the family Ostreidae. II. Interspecific studies of the genera *Crassostrea* and *Saccostrea. Marine Biology,* 54: 171–184.

CARLSON, S. S., WILSON, A. C. & MAXSON, L. D., 1978, Do albumin clocks run on time? A reply. *Science,* 200: 1183–1185.

CARSON, H. L., 1976, Inference of the time of origin of some *Drosophila* species. *Nature,* 259: 395–396.

CAVALLI-SFORZA, L. L. & EDWARDS, A. W. F., 1967, Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics,* 19: 233–257.

CHERRY, L. M., CASE, S. M. & WILSON, A. C., 1978, Frog perspective on the morphological difference between humans and chimpanzees. *Science,* 200: 209–211.

DARLINGTON, P. J., Jr., 1957, *Zoogeograpy: the geographical distribution of animals.* Wiley, New York, 675 p.

DOBZHANSKY, Th., 1970, *Genetics of the evolutionary process.* Columbia University Press, New York, 505 p.

GALTSOFF, P. S., 1964, The American oyster *Crassostrea virginica* Gmelin. *United States Department of the Interior Fishery Bulletin of the Fish and Wildlife Service,* 64: iii + 480 p.

GROUE, K. J. & LESTER, L. J., 1982, A morphological and genetic analysis of geographic variation among oysters in the Gulf of Mexico. *Veliger,* 24: 331–335.

HERTLEIN, L. G., 1951, Description of two new species of marine pelecypods from West Mexico. *Bulletin of the Southern California Academy of Sciences,* 50: 68–75.

HILLMAN, R. E., 1964, Chromatographic evidence of intraspecific genetic differences in the eastern oyster, *Crassostrea virginica. Systematic Zoology,* 13: 12–18.

HILLMAN, R. E., 1965, Chromatographic studies of allopatric populations of the Eastern oyster, *Crassostrea virginica. Chesapeake Science,* 6: 115–116.

JOHNSON, A. G., UTTER, F. M. & NIGGOL, K., 1972, Electrophoretic variants of aspartate amino transferase and adductor muscle proteins in the native oyster (*Ostrea lurida*). *Animal Blood Groups and Biochemical Genetics,* 3: 109–113.

KEIGWIN, L., 1982, Isotopic paleoceanography of the Caribbean and East Pacific: role of Panama uplift in late Neogene time. *Science,* 217: 350–353.

KORRINGA, P., 1952, Recent advances in oyster biology. *Quarterly Review of Biology,* 27: 266–308, 339–365.

LESSIOS, H. A., 1979, Use of Panamanian sea urchins to test the molecular clock. *Nature,* 280: 599–601.

LESSIOS, H. A., 1981, Divergence in allopatry: molecular and morphological differentiation between sea urchins separated by the Isthmus of Panama. *Evolution,* 35: 618–634.

LI, M. F., FLEMING, C. & STEWART, J. E., 1967, Serological differences between two populations of oysters (*Crassostrea virginica*) from the Atlantic coast of Canada. *Journal of the Fisheries Research Board of Canada,* 24: 443–446.

LONGWELL, A. C., STILES, S. S. & SMITH, D. G., 1967, Chromosome complement of the American oyster *Crassostrea virginica* as seen in meiotic and cleaving eggs. *Canadian Journal of Genetics and Cytology,* 9: 845–856.

MATTOX, N. T., 1949, Studies on the biology of the edible oyster, *Ostrea rhizophorae* (Guilding) in Puerto Rico. *Ecological Monographs,* 19: 330–356.

MENZEL, R. W., 1951, Early sexual development and growth of the American oyster in Louisiana waters. *Science,* 113: 719–721.

MENZEL, R. W., 1956, The effect of temperature on the ciliary action and other activities of oysters. *Florida State University Studies,* 22: 25–36.

MENZEL, R. W., 1968a, Chromosome number in nine families of marine pelecypod mollusks. *Nautilus,* 82: 45–50, 53–58.

MENZEL, R. W., 1968b, Cytotaxonomy of species of clams (*Mercenaria*) and oysters (*Crassostrea*). *Proceedings of the Symposium on Mollusca, Marine Biological Association of India,* Part I: 75–84.

MENZEL, R. W., 1971, Possibilities of molluscan cultivation in the Caribbean. *FAO Fisheries Resources Division Report,* 71.2: 183–200.

MENZEL, R. W., 1973, Some species affinities in the oyster genus *Crassostrea. Bulletin of the American Malacological Union,* 1973: 38.

NEI, M., 1971, Interspecific gene differences and evolutionary time estimated from electrophoretic data on protein identity. *American Naturalist,* 105: 385–398.

NEI, M., 1975, *Molecular population genetics and evolution.* American-Elsevier, New York, 288 p.

NEI, M., 1978, Estimation of average heterozygos-

ity and genetic distance from a small number of individuals. *Genetics,* 89: 583–590.

NEI, M. & ROYCHOUDHURY, A. K., 1974, Sampling variances of heterozygosity and genetic distance. *Genetics,* 76: 379–390.

NUMACHI, K., 1962, Serological studies of species and races of oysters. *American Naturalist,* 96: 211–217.

RODRIGUEZ-ROMERO, F., LAGUARDA-FIGUERAS, A. & URIBE-ALCOCER, M., 1979b, Comparative analysis of the karyotypes of two oyster species of the genus *Crassostrea* from Mexico: *C. virginica* and *C. corteziensis. Anales de Centro de Ciencias del Mar y Limnologia, Universidad Nacional Autonoma de Mexico,* 6(1): 19–24.

RODRIGUEZ-ROMERO, F., URIBE-ALCOCER, M. & LAGUARDA-FIGUERAS, A., 1978, Cytogenetic study of an oyster population, of the species, *Crassostrea virginica* Gmelin, from the coasts of Tabasco, Mexico. *Venus, Japanese Journal of Malacology,* 37: 83–86.

RODRIGUEZ-ROMERO, F., URIBE-ALCOCER, M., LAGUARDA-FIGUERAS, A. & DIUPOTEX-CHONG, M. E., 1979a, The karyotype of *Crassostrea rhizophorae* (Guilding, 1828). *Venus, Japanese Journal of Malacology,* 38: 135–140.

SELANDER, R. K., 1970, Behavior and genetic variation in natural populations. *American Zoologist,* 10: 53–66.

SELANDER, R. K., SMITH, M. H., YANG, S. Y., JOHNSON, W. E. & GENTRY, J. B., 1971, Biochemical polymorphism and systematics in the genus *Peromiscus.* I. Variation in the old-field mouse. *Studies in Genetics, University of Texas Publications* 7103, VI: 49–90.

SINGH, S. M. & GREEN, R. H., 1984, Excess of allozyme homozygosity in marine molluscs and its possible biological significance. *Malacologia,* 25: 569–581.

SNEATH, P. H. A. & SOKAL, R. R., 1973. *Numerical taxonomy.* Freeman, San Francisco, 573 p.

STAUBER, L. A., 1947, On possible physiological species in the oyster, *Ostrea virginica. Anatomical Record,* 94: 614.

STAUBER, L. A., 1950, The problem of physiological species with special reference to oysters and oyster drills. *Ecology,* 31: 109–118.

STENZEL, H. B., 1971, Oysters. In: MOORE, R. C., ed., *Treatise on Invertebrate Paleontology.* Geological Society of America, and University of Kansas, Part N, Vol. 3 (of 3), Mollusca 6: N953–N1224.

SWOFFORD, D. L., 1981, On the utility of the distance Wagner procedure. In: FUNK, V. A. & BROOKS, D. R., eds., *Advances in Cladistics: Proceedings of the First Meeting of the Willi Hennig Society,* New York Botanical Garden, N.Y., p. 25–43.

SWOFFORD, D. L. & SELANDER, R. B., 1981, BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity,* 72: 281–283.

TRACEY, M. L., NELSON, K., HEDGECOCK, D., SHLESER, R. A. & PRESSICK, M. L., 1975, Biochemical genetics of lobsters (*Homarus*): genetic variation and the structure of American lobster populations. *Journal of the Fisheries Research Board of Canada,* 32: 2091–2101.

VAWTER, A. T., ROSENBLATT, R. & GORMAN, G. C., 1980, Genetic divergence among fishes of the eastern Pacific and Caribbean: support for the molecular clock. *Evolution,* 34: 705–711.

WARMKE, G. L. & ABBOTT, R. T., 1961, *Caribbean seashells.* Dover, New York, 348 p.

WILSON, A. C., 1976, Gene regulation in evolution. In: AYALA, F. J., ed., *Molecular evolution.* Sinauer, Sunderland, Massachusetts, p. 225–234.

WILSON, A. C., CARLSON, S. S. & WHITE, T. J., 1977, Biochemical evolution. *Annual Review of Biochemisry,* 46: 473–639.

WILSON, A. C., MAXSON, L. R. & SARICH, V. M., 1974a, Two types of molecular evolution. Evidence from studies of interspecific hybridization. *Proceedings of the National Academy of Science, U.S.A.,* 71: 2843–2847.

WILSON, A. C., SARICH, V. M. & MAXSON, L. R., 1974b, The importance of gene rearrangement in evolution: evidence from studies on rates of chromosomal, protein, and anatomical evolution. *Proceedings of the National Academy of Science, U.S.A.,* 71: 3028–3030.

WILSON, A. C., BUSH, G. L., CASE, S. M. & KING, M.-C., 1975, Social structuring of mammalian populations and rate of chromosomal evolution. *Proceedings of the National Academy of Science, U.S.A.,* 72: 5061–5065.

WRIGHT, S., 1943, Isolation by distance. *Genetics,* 28: 114–138.

YANG, S. Y., SOULE, M. & GORMAN, G. C., 1974, *Anolis* lizards of the eastern Caribbean: a case study in evolution. I. Genetic relationships, phylogeny and colonization sequence of the *roquet* group. *Systematic Zoology,* 23: 387–399.

ZOUROS, E. & FOLTZ, D. W., 1984, Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia,* 25: 583–591.

MALACOLOGIA, 1984, 25(2): 551–568

THE SYSTEMATIC STATUS OF *MYTILUS GALLOPROVINCIALIS* IN WESTERN EUROPE: A REVIEW

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ABSTRACT

The controversy concerning the systematic status of the Mediterranean mussel *Mytilus galloprovincialis* Lmk. dates back to the 1860s. While some authorities regard *M. galloprovincialis* as a distinct species, others consider it a variety of *Mytilus edulis* L. Separation of *M. edulis* and *M. galloprovincialis* has traditionally been based primarily on morphological shell characteristics. The first part of this paper discusses the reliability of these characters in separating mixed samples of the two types of mussel in W Europe. The conclusion is that with few exceptions—e.g. Rock in SW England, where there is some evidence of premating isolating mechanisms—separation based solely on shell morphology is unreliable, especially in areas where extensive hybridization and introgression is taking place.

In recent years, techniques such as gel electrophoresis, cytology, immunology and artificial hybridization have been used in conjunction with shell morphology in an attempt to resolve the problem of the taxonomic status of *M. galloprovincialis*. The second and major part of this paper reviews the results of such studies. Data from electrophoretic surveys has shown that the two forms of mussel are closely related; genetic identity and genetic distance values are similar to those observed between subspecies of other invertebrates. Cytological and immunological studies on *M. edulis* and *M. galloprovincialis* indicate that the differences between them are small and hardly sufficient to warrant them being classified as separate species. Results from hybridization studies have probably contributed most to a resolution of the problem; there is no evidence of genetic incompatibility between the two forms since the hybrids are fertile and viable backcross F_2 individuals have been produced.

Thus, while the differences between the two forms of mussel are greater than between geographically isolated populations of most species, they are hardly large enough to justify *M. galloprovincialis* being considered a distinct species. The conclusion from this review is that *M. galloprovincialis* cannot be regarded as more than a race or subspecies of *M. edulis* L. and it is suggested that the name *Mytilus edulis* var. *galloprovincialis* be readopted, the ranking suggested by Jeffreys (1863).

Key words: mussels; *Mytilus*; morphological variations; population genetics; cytogenetics; immunology; hybridization; systematics.

INTRODUCTION

The systematic status of *Mytilus galloprovincialis* Lmk. has been the subject of considerable discussion since the 1860s. While some authorities regard it as a distinct species of *Mytilus* others regard it merely as a variety of the larger *Mytilus edulis* L. complex (review, Lubet, 1973).

M. galloprovincialis is believed to have originated in the Mediterranean area (Barsotti & Meluzzi, 1968) but evidence now indicates that it has extended its range northwards onto the Atlantic coasts of W Europe, where it is found intermixed with *M. edulis* in varying proportions (Hepper, 1957; Seed, 1972; Lubet, 1973; Seed, 1974).

The two forms of mussel have been traditionally separated on the basis of shell morphology but in some areas, e.g. the Atlantic coasts of Ireland and NW France where there is evidence of extensive hybridization (Seed, 1972, 1974; Gosling & Wilkins, 1977; Skibinski & Beardmore, 1979; Gosling & Wilkins, 1981), identification on shell characters alone is difficult and even impossible. In recent years the technique of gel electrophoresis (in conjunction with morphological analyses) has been used in an attempt to quantify the genetic differences between the two forms (Ahmad & Beardmore, 1976; Gosling & Wilkins, 1977; Skibinski *et al.*, 1978b; Skibinski *et al.*, 1980; Gosling & Wilkins, 1981).

The first part of this paper discusses the

reliability of the morphological criteria used to separate *M. galloprovincialis* from *M. edulis*, while the second part assesses the contribution that gel electrophoresis and other techniques such as cytological, immunological and artificial hybridization studies have made towards a better understanding of the systematics of *Mytilus* in W Europe.

MORPHOLOGICAL CRITERIA USED TO SEPARATE *M. EDULIS* AND *M. GALLOPROVINCIALIS*—AN EVALUATION

Separation of the two forms of mussels is based primarily on external shell contours, internal features of the shell valves and the colour of the mantle edge. Although detailed descriptions of these can be found elsewhere (Lewis & Powell, 1961; Lewis & Seed, 1969; Seed, 1972; Lubet, 1973; Seed, 1974, 1978) a brief account of the reported distinguishing characters will be included at this point.

The shell of *M. galloprovincialis* tends to be higher and flatter than in *M. edulis*, giving distinctly different transverse profiles in the two forms (Fig. 1B). The anterior end of the shell of *M. galloprovincialis* is distinctly beaked or incurved while that of *M. edulis* has a more snub-nosed appearance. In *M. galloprovincialis* the anterior edge of the shell merges smoothly into the dorsal edge, thus giving rise to a rounded convex profile, while that of *M. edulis* is angular where the anterior and dorsal edges meet (Fig. 1A).

Interiorly, the anterior adductor muscle scar is small and circular in *M. galloprovincialis* whereas in *M. edulis* it is narrow and elon-

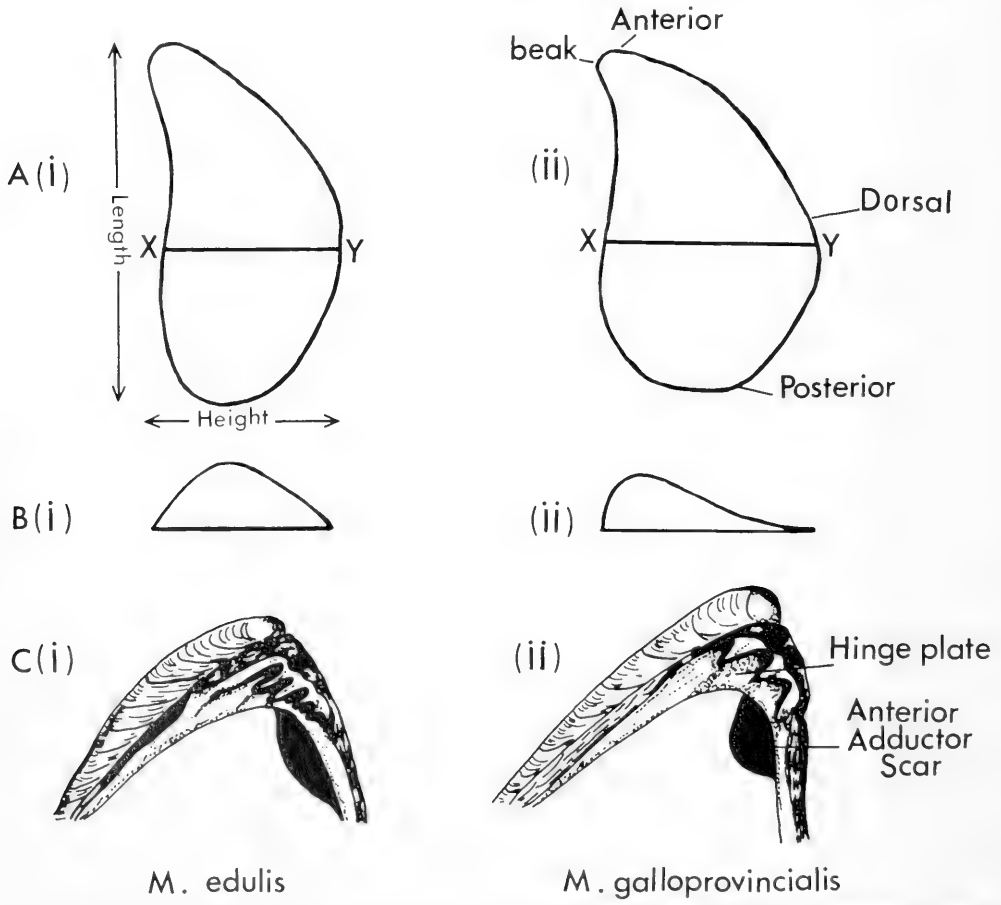

FIG. 1. A (i) and (ii) and C (i) and (ii): some of the salient differences in shell morphology between *M. edulis* and *M. galloprovincialis*. B (i) and (ii): transverse profiles through section XY of a single shell valve of *M. edulis* and *M. galloprovincialis*.

gated. The hinge plate is also smaller in *M. galloprovincialis*, forming a much tighter arc with its rear end much more clearly delimited from the adjacent ventral edge of the valve. The hinge plate in *M. edulis* is a gently curving structure. Both of these characters are illustrated in Fig. 1C. The colour of the mantle edge tends to be deep purple-violet in *M. galloprovincialis* and yellow-brown in *M. edulis*.

Using these characters a number of investigators (Lewis & Seed, 1969; Seed, 1972, 1974) have identified the *M. galloprovincialis* form on the SW coasts of England and on the Atlantic coasts of Ireland and France extending into the English Channel as far as the Cherbourg peninsula. In these areas *M. galloprovincialis* has been found intermixed with *M. edulis* in varying proportions. In some areas, e.g. the Atlantic coasts of Ireland and NW France, separation of the two forms of mussel has proved to be exceedingly difficult due to a considerable degree of overlap in morphological characteristics. This, together with the large numbers of truly intermediate forms observed, suggests that hybridization might be occurring (Seed, 1978).

In other areas, e.g. SW England and the Bay of Biscay (France), the task of separation, although less difficult, was confounded by the fact that shell morphology is so enormously plastic, being influenced by such factors as age and density of mussels, tidal level, and habitat type (Seed, 1968). The effects of these factors appear to be the same for both forms of mussel (Seed, 1978) and this results in a considerable degree of convergence in mixed samples. For example, when the two forms of mussels, together with their hybrids (all spawned artificially) were grown in the wild under identical conditions of temperature, salinity, nutrition, photoperiod and population density, Lubet *et al.* (1984) found that shell shape could not be used to distinguish *M. edulis* from *M. galloprovincialis* or their hybrids.

I will now consider which, if any, of the morphological characters variously attributed to *M. galloprovincialis* are sufficiently constant to distinguish it from *M. edulis* as found on the coasts of Britain, France and Ireland.

External Shell Morphology

Both *M. edulis* and *M. galloprovincialis* exhibit considerable variation in external shell morphology and of the two *M. galloprovincialis* seems to be the more variable form. Seed (1972), in a detailed morphological survey of mussels from sixteen locations on the French coasts, points out that "over 30% of all the mussels examined during this investigation would have been misidentified on external characters alone." Similar problems of identification have been encountered in SW England (Lewis & Seed, 1969). In a survey of Irish mussel populations, Seed (1974) found gross shell morphology to be completely unreliable in separating the two forms; mussels of every conceivable shape were encountered from one locality to another. It would appear, therefore, that overall shell shape in *Mytilus* is so variable, both within and between the two forms of mussels, that it has little if any value in taxonomic studies.

Internal Shell Morphology

The anterior adductor scar and hinge plate size have generally been regarded as more reliable taxonomic characters in the separation of the two forms (Seed, 1978). While the mean adductor scar ratios (adductor scar length/shell length) vary from one locality to another the values tend to be consistently lower in *M. galloprovincialis* than in *M. edulis*. In Ireland, Seed (1974) found the range to be 53–93 for *M. galloprovincialis* and 74–113 for *M. edulis*; in France (Seed, 1972) 41–87 (*M. galloprovincialis*) and 80–124 (*M. edulis*) and at Rock, SW England (Lewis & Seed, 1969) the mean values were 64 ± 10 (*M. galloprovincialis*) and 105 ± 13 (*M. edulis*). However, there are difficulties associated with using this character as the sole means of distinguishing between the two forms. For example, on the west coast of France, Lubet (1959) and Seed (1972) have found considerable overlap in values and distributions were not markedly bimodal; while at sites on the Mediterranean coast—where the *M. edulis* form is very rare—the distribution was distinctly skewed. The percentage misidentification using this character alone was as high as 45% for areas on the west coast of France and over 60% for several localities in the Mediterranean Sea.

Like the adductor scar ratios, the hinge plate ratios (hinge plate length/shell length)—although difficult to measure accurately (Lewis & Seed, 1969)—tend to be lower in *M. galloprovincialis* than in *M. edulis*: 74–77 (*M. galloprovincialis*) and 70–115 (*M. edulis*) in Ireland; 51–70 (*M. galloprovincialis*) and 56–99 (*M. edulis*) in France; 56 ± 8 (*M. gallopro-*

vincialis) and 72 ± 9 (*M. edulis*) at Rock. Once again there are difficulties with the use of this character as a sole means of distinguishing between the two forms because the degree of overlap is such that for the majority of French sites (Seed, 1972) and for Rock (Lewis & Seed, 1969) the frequency distributions of the hinge plate ratios were generally unimodal for mixed samples of the two forms of mussel. In the Mediterranean, 33% of mussels from Sète would have been identified as *M. edulis* using this character alone.

Because hinge plate size has limited usefulness as a means of separating mixed populations, Seed (1972) suggests that hinge plate *shape* should also be considered. But the shape of the hinge plate is a difficult character to assess, especially in upper shore mussels where the shape of the anterior end is obscured by erosion, shell thickening and divergence of umbones (Lewis & Seed, 1969).

In contrast to the situation observed in W France the hinge plate ratios in Irish samples generally proved to be a more reliable taxonomic character than the adductor scar ratios, with frequency distributions in some cases quite distinctly bimodal (Seed, 1974). The percentage of mussels misidentified using hinge plate ratios was considerably lower than for adductor scar ratios, i.e. 14% and 50% respectively for an exposed site (Portstewart) on the N coast of Ireland.

Mantle Edge Colour

This character has been cited by Seed (1972) as being generally a reliable taxonomic character for French populations of mussels. This is surprising in view of his comment that "mantle edge colour varied from almost white in some individuals through all shades of brown, reddish-brown and purple to deep-violet in others." In addition, in the Concarneau-Les-Sables region on the W coast of France between 20–45% of the populations would have been misidentified using mantle edge colour alone. More remarkably still, the value of this character completely breaks down in samples from Banyuls, Sète and Riou on the Mediterranean coast of France where over 60% of mussels would have been identified as *M. edulis* using this character! In SW England, Lewis & Powell (1961) observed dark mantle types with typical *M. edulis* characters at all sites, while in Ireland Seed (1974) observed "individuals with characteristic *galloprovincialis*-like shells with mantles that were distinctly pale in colour." Certainly in Ireland the value of mantle colour as a diagnostic taxonomic character is questionable since at 18 of the 37 Atlantic sites sampled more than 20% of individuals were misidentified using this character and at some sites the misidentification percentage was as high as 46%.

In conclusion, there is no single character which can be reliably used to separate the two forms of mussels in W Europe. In some instances, e.g. at Rock, it appears that using a combination of characters, such as adductor and hinge plate ratios together with mantle edge colour, gives a high degree of accuracy. Moreover, at Rock, morphological and anatomical differences were also accompanied by marked differences in breeding patterns, peacrab infestation and growth potential (Seed, 1971). Sites such as Rock, where the two mussel types are quite distinctive are of especial interest and obviously require further study. Lewis & Seed (1969), using a combination of morphological and anatomical characters at a number of other sites in SW England did not, however, produce identical results (see Table 1). The same inconsistencies were observed by Seed (1972) for mixed populations of the two forms of mussels in W France. Furthermore, in areas such as the Atlantic coasts of Ireland and NW France, where hybridization is taking place, the large number of intermediate forms makes accurate identification of *M. galloprovincialis* and *M. edulis* an almost impossible task especially at exposed locations (Seed, 1974).

THE CONTRIBUTION OF ELECTROPHORESIS, CYTOLOGY, IMMUNOLOGY AND ARTIFICIAL HYBRIDIZATION TO THE SYSTEMATICS OF *MYTILUS*

Electrophoretic Studies

Morphological studies do not fully take into account that the environment can substantially influence the morphological characteristics of a given species. Therefore, systematic information that is relatively free of environmentally-induced changes is highly desirable. Protein electrophoresis together with techniques such as DNA-RNA hybridization, immunology and amino acid sequencing

TABLE 1. A comparison of identifications based on mantle colour, overall shell shape and hinge and adductor scar ratios in samples of *Mytilus* from SW England (after Lewis & Seed, 1969).

		Method of identification						
		Mantle colour		Shell shape			Hinge and add. scar	
Locality	Sample size	*gall.*	*edulis*	*gall.*	?	*edulis*	*gall.*	*edulis*
Penzance Harbour	20	9	11	2	5	13	6	14
St. Ives	62	62	—	25	10	27	53	9
Bude low shore	44	44	—	11	20	13	36	8
Bude high shore	70	?	?*	18	11	41	52	18

*Mantle colour appeared too dark for *M. edulis* and not purple enough for *M. galloprovincialis*.

have proved invaluable in quantifying genetic differences between species. For comparisons of closely related species, electrophoresis has proved to be a most efficacious technique (see Ferguson, 1980 for review and references). "In most cases where the status of a 'species' is in dispute," as is the case for *M. galloprovincialis*, "an electrophoretic investigation of enzyme loci is likely not only to produce an answer less open to argument than one based on conventional morphological criteria, but it is able to produce results more quickly" (Thorpe *et al.,* 1978).

Genetic variability at several enzyme loci has been surveyed in allopatric populations of pure *M. edulis* from approximately 75 sites on the coasts of Ireland and France (Gosling & Wilkins, 1977, 1981), Britain (Ahmad *et al.,* 1977), and Denmark (Theisen, 1978). In general, there appears to be very little geographic variation in allele frequency with the exception of the Baltic area where Theisen (1978) has observed conspicuous differences in allele frequency at three enzyme loci between the North Sea and Kattegat *M. edulis* and those from the Baltic. Salinity is believed to be the selective agent maintaining these differences since the maximum change in allele frequency, at the three loci, roughly coincides with the transition from the rather stable low salinities of the Baltic (7–10‰) to the higher more variable salinities of the Kattegat (22–30‰).

In contrast, pure populations of *M. galloprovincialis* have been analysed electrophoretically from only a small number of sites (about four) in the Mediterranean and Black Sea. Allele frequency data from 12 enzyme loci (Skibinski *et al.,* 1980) at two sites (Venice and Gibraltar) indicate that there are large differences in frequency at several loci (Aminopeptidase (*Ap*), leucine aminopeptidase-1 (*Lap-1*), phosphoglucomutase (*Pgm*), and phosphoglucose isomerase(*Pgi*). However, Nei's genetic identity value (Nei, 1972) for the two populations is 0.976, which is in the range expected for conspecific populations. Although genetic identity values have not been published for *M. edulis* populations the indications are that they would be similar (if not higher) than the above value for *M. galloprovincialis*. Nei's (1972) genetic identity ($\bar{I}$) and genetic distance ($\bar{D}$) values for comparisons between *M. edulis* from S Wales and *M. galloprovincialis* from Venice were found to be 0.842 and 0.167 respectively (Skibinski *et al.,* 1980). These values are similar to the mean values observed for comparisons between subspecies of other invertebrate taxa (Snyder & Gooch, 1973; Ayala *et al.,* 1974; Avise, 1976). Large $\bar{I}$ values (>0.90) have been observed, however, for comparisons of distinct species of *Elliptio* (Davis *et al.,* 1981), *Drosophila* (Sene & Carson, 1977), *Partula* (Johnson *et al.,* 1977) and *Cerion* (Gould & Woodruff, 1978), thus illustrating the dangers associated with inferring taxonomic relationships from $\bar{I}$ or $\bar{D}$ values alone. Using a band-counting electrophoretic method (see Ferguson, 1980 for description of technique) ten individuals of *M. edulis* (W Ireland) and *M. galloprovincialis* (S France) have been compared on a single gel; the coefficient of similarity between them was 0.809 (Gosling & King, unpublished results), a value very close to that observed by Skibinski *et al.* (1980)

TABLE 2. Allele frequencies at *Est-D, Lap-1* and *Pgi* for *M. edulis* at Mumbles, S Wales and *M. galloprovincialis* at Venice. E and G are the two compound alleles at each locus. Alleles are numbered in order of increasing anodal mobility. N – number of individuals analysed. Data from Skibinski *et al.* (1980).

	N	1	2	3	4	5	6	7
Est-D		G				E		
M. edulis	1286	0.005	0.024	0.001	0.941	0.004	0.020	0.003
M. gall.	120	0.038	0.950	0.0	0.013	0.0	0.0	0.0
Lap-1		E				G		
M. edulis	1286	0.019	0.198	0.724	0.006	0.049	0.004	0.0
M. gall.	31	0.0	0.0	0.032	0.032	0.468	0.043	0.032
Pgi		G				E		
M. edulis	2005	0.013	0.033	0.247	0.049	0.599	0.048	0.011
M. gall.	226	0.009	0.022	0.801	0.142	0.024	0.0	0.022

using allele frequency data from 16 enzyme loci. Sarich (1977) has found positive agreement between the two methods for interspecific comparisons of *Dipodomys* (kangaroo rat).

The two forms of mussel differ most at the following enzyme loci: Esterase-D (*Est-D*), *Lap-1, Pgi, Ap, Lap-2* and *Pgm* with the first three being partially diagnostic (a locus is considered to be diagnostic if an individual can be assigned to the correct species (or form) with a probability >0.99 (Avise, 1974)). The search for diagnostic loci, where the allele frequency distributions do not overlap, has to date been unsuccessful (Skibinski *et al.,* 1978b). Table 2 presents typical allele frequency data at the *Est-D, Lap-1* and *Pgi* loci for *M. edulis* (S Wales) and *M. galloprovincialis* (Venice). One must be cautious however about accepting that 'typical' frequencies for *M. galloprovincialis* exist since so few populations have been analysed and since moderate to large differences in allele frequency have been observed at several loci (see above). The allele frequency distributions at the three loci are significantly different between the two mussel types; the differences are greater at the *Est-D* and *Lap-1* loci than at the *Pgi* locus. Skibinski *et al.* (1978b) have suggested combining alleles at each of these loci to form compound (synthetic) alleles called E and G. The Est^E (alleles 4, 5, 6 and 7), Lap^E (alleles 1, 2, 3 and 4) and Pgi^E (alleles 5 and 6) compound alleles combine those alleles at high frequency in *M. edulis* while the Est^G (alleles 1, 2 and 3), Lap^G (5, 6 and 7) and Pgi^G (1, 2, 3 and 4) compound alleles combine those at high frequency in *M. galloprovincialis* (see Table 2). This procedure smooths out allele frequency differences between populations of *M. edulis* or *M. galloprovincialis.* For example, the frequencies of alleles 3 and 4 at the *Pgi* locus were 0.544 and 0.344 respectively in *M. galloprovincialis* from Gibraltar and 0.801 and 0.142 in *M. galloprovincialis* from Venice. However, the frequency of the compound allele G is 0.951 for Gibralter and 0.974 for Venice.

In summary, electrophoretic investigations on allopatric populations of the closely related *M. edulis* and *M. galloprovincialis* have revealed little geographic heterogeneity in allele frequency in *M. edulis* but a moderate amount in *M. galloprovincialis.* When the two forms are compared they differ significantly in allele frequency at the *Est-D, Lap-1, Pgi, Ap, Lap-2* and *Pgm* loci with the first three being partially diagnostic.

This information has been used to study the genetic structure of mixed populations of the two forms of mussels at British and Irish coastal sites. In Britain, Hepper (1957) reported the presence of a form of mussel, "the Padstow mussel"—which corresponded closely to the description of *M. galloprovincialis*—on the coasts of SW England. Later investigations (Seed, 1971) indicated that the two forms of mussels in that area were morphologically quite distinct, had different spawning cycles, growth rates and infection rates with *Pinnotheres pisum* (pea-crab); on the basis of these differences Seed sug-

TABLE 3. Allele frequencies at *Pgi* in *M. edulis* (Group 1) and the "Padstow mussel" (Group 2) from Rock, SW England (data from Ahmad & Beardmore, 1976). Data from Skibinski *et al.* (1980) have been included for comparative purposes.

		G				E		
	N	1	2	3	4	5	6	7
M. edulis (S Wales)	2005	0.013	0.003	0.247	0.049	0.599	0.048	0.011
Group 1	100	0.005	0.070	0.365	0.125	0.410	0.025	0.0
Group 2	100	0.025	0.035	0.460	0.390	0.080	0.010	0.0
M. gall. (Venice)	226	0.009	0.022	0.801	0.142	0.024	0.0	0.022

gested that the two types of mussel should be regarded as distinct species of *Mytilus*. At this stage no firm genetic evidence had been gathered on the two forms of mussels but the morphological and physiological differences suggested that there were good grounds for biochemical investigations.

Ahmad & Beardmore (1976), using the morphological characteristics already discussed, separated mussels from Rock, S.W. England, into *M. galloprovincialis* and *M. edulis* forms and assayed each group for *Pgi, Pgm, Lap-2* and *Ap* (the electrophoretic techniques for *Est-D* and *Lap-1* had not yet been developed). The results indicated significant differences in allele frequency between the two groups at the *Pgi, Ap* and *Lap-2* loci but not at the *Pgm* locus. On the basis of these differences, together with the close similarity in allele frequency between the *M. galloprovincialis* form at Rock and *M. galloprovincialis* from the Mediterranean, the authors concluded that the "Padstow mussel" was indeed *M. galloprovincialis*. In addition, they felt that the two groups at Rock were genetically discrete and reproductively isolated entities since no genetic evidence for hybridization was observed. Allele frequencies at the *Pgi* locus (the two groups showed the biggest difference in frequency at this locus) for the two types of mussel are presented in Table 3. Allele frequency data for *M. edulis* (S Wales) and *M. galloprovincialis* (Venice) have been included for comparative purposes (data from Skibinski *et al.*, 1980). While the *M. edulis* and *M. galloprovincialis* group differed significantly at the *Pgi* locus it would appear from Table 3 that there are large differences in allele frequency between *M. edulis* from Rock and *M. edulis* from S Wales. Ahmad & Beardmore (1976) suggested that in British populations of *M. edulis* there may be a N–S cline in the frequency of Pgi^3 and Pgi^5. However, subsequent analysis has indicated little geographic heterogeneity in allele frequency at the *Pgi* locus among several British populations of *M. edulis* (Ahmad *et al.*, 1977). It may be that the *M. edulis* group was not a homogeneous group but contained some *M. galloprovincialis* forms. Ahmad & Beardmore (1976) did have some difficulties in the separation of the two forms of mussel as the following illustrates. The remainder of the sample from Rock (187 individuals) was assayed for *Pgi* and allele frequencies resembled—but were not identical—to those observed for the *M. galloprovincialis* group (using the compound alleles E and G one can compare the number of E/G heterozygotes observed with Hardy-Weinberg expectations (see later); there is a significant deficiency of heterozygotes ($P < 0.001$) in this sample which indicates that it is in fact a mixture of populations with differing allele frequency). After electrophoretic analysis Ahmad & Beardmore (1976) examined the 187 individuals morphologically and found that the majority (155) resembled the 'Padstow mussel' (Group 2) but 32 individuals could not be classified at all. However, genetically they were similar to *M. edulis* (Group 1). This illustrates once more the difficulties of distinguishing between these two forms of mussels using morphological criteria.

The Rock site was revisited by Skibinski *et al.* (1978a), and mussels were collected from an area close to the one sampled by Ahmad & Beardmore (1976). In addition, mussels were collected from an exposed rocky shore at Croyde, N Devon. The samples were divided into *M. galloprovincialis*, *M. edulis* and intermediates but this time a scoring system for the individual morphological characters was used with typical *M. edulis* getting low scores, typical *M. galloprovincialis* high scores and the intermediates scoring between the two extremes. However, there were difficulties with the scoring method. The authors observed "some phenotypic overlap for in-

dividual morphological characters between pure *M. edulis* and pure *M. galloprovincialis* populations (from Ilfracombe, N. Devon and Venice) so intermediate scores *are expected* [my italics] in the absence of hybridization." The following proportions were observed at the two sites:

	% *M. edulis*	Intermediates	*M. galloprovincialis*
Rock	1	14	85
Croyde	65	33	2

Each group was assayed for the three partially diagnostic loci and significant differences in allele frequency at the three loci were observed between *M. galloprovincialis* and *M. edulis* at both sites. Allele frequency for the intermediates fell between the frequencies observed for the extremes (intermediates this time appear to be truly intermediate and not *M. edulis* individuals as observed by Ahmad & Beardmore (1976)). In general, the frequencies of *M. edulis* and *M. galloprovincialis* corresponded with those observed for allopatric populations of the two forms. This was particularly true for Rock *M. edulis* which on this occasion were very similar in allele frequency at the *Pgi* locus to *M. edulis* from S Wales. This probably reflects better separation of the two forms using a scoring system.

As described above, the frequencies of alleles at each locus were pooled to form compound alleles E and G for the total sample of mussels from each site. Analysis of the data revealed:

a) A large and significant deficit of E/G heterozygotes compared with Hardy-Weinberg expectations in both samples, which was to be expected when sampling a mixture of populations, each with differing allele frequency.

b) A significant excess of triple homozygotes in the Rock sample and a significant excess of double homozygotes in the sample from Croyde (*Pgi* was not studied at Croyde). This occurs because the frequency of $Est\text{-}D^E$ $Est\text{-}D^E$ $Lap\text{-}1^E$ / $Lap\text{-}1^E$ Pgi^E / Pgi^E homozygotes covary positively between contributing populations; the same holds true for G/G homozygotes.

c) An overall excess of triple heterozygotes at Rock and an excess of double heterozygotes at Croyde; the excess was higher among morphologically intermediate mussels. This has been interpreted as evidence for hybridization since "If at a locus the alleles of highest frequency are different in two species, a population containing a significant number of F_1 hybrids will show an excess of heterozygotes compared with the expectation for either species or for a mixed population of the two pure species. The excess will be relatively greater for multiple heterozygotes at two or more diagnostic or discriminatory loci" (Skibinski *et al.*, 1978a). The amount of hybridization was calculated as 2% for Rock and 5% for Croyde. In addition, a significant number (21%) of 'F_2' and 'backcross' genotypes were observed in the intermediate group at Croyde but not at Rock.

Subsequently, Skibinski *et al.* (1978b) investigated mussel populations at Robin Hood's Bay (RHB) on the NE coast of England and at King's Dock in S Wales. The data from the Croyde site was also included in that paper. Mussels were again separated on the basis of the morphological criteria outlined by Lewis & Seed (1969) and the same scoring system as before was used. Once again difficulties were encountered in identifying *M. galloprovincialis* and *M. edulis* individuals. At Croyde and RHB typical *M. galloprovincialis* and *M. edulis* were identified (this was the first report of *M. galloprovincialis* in the North Sea) with the best discriminating characters being the colour of the mantle edge and the size and shape of the adductor scar. However, intermediates and individuals showing all combinations of *M. edulis* and *M. galloprovincialis* characteristics were present. These two characters were of little use at King's Dock and while most of the mussels at that site were identified on other characteristics as *M. galloprovincialis*, they had a large adductor scar and the yellow mantle edge of the *M. edulis*. It is not surprising that Skibinski *et al.* (1978b) felt that "quantification of the morphological variation within and between populations is certainly desirable"! The morphological extremes—which represented a much smaller fraction (5–26%) of the total sample in each case—were typed for *Est-D* and *Lap-1* and the data were treated in the same way as described in Skibinski *et al.* (1978a)—see above. The results for the three sites are summarized in Table 4. Skibinski *et al.* (1978b) conclude from these results that the extent of mixing is low at Croyde (but see above), intermediate at RHB and high at King's Dock. It would appear from the genetic

TABLE 4. Summary of results for *Lap-1* and *Est-D* in mixed samples of mussels from Croyde, Robin Hood's Bay (RHB) and King's Dock [data from text of Skibinski *et al.*, 1978b]. ** = $P < 0.01$; *** = $P < 0.001$; n.s. not significant.

	Croyde	RHB	King's Dock
Allele frequency differences between morphological extremes	Large	Smaller than Croyde	None
Heterozygote deficits			
Lap-1	***	***	n.s.
Est-D	***	**	n.s.
Genotype Association			
Excess of double homozygotes	***	***	n.s.
Excess of double heterozygotes	***	n.s.	n.s.

evidence so far that at all of the sites where the two forms of mussel were found together, hybridization and introgression to varying extents were taking place between them.

Let us now consider the situation in Ireland. In 1974, Seed made extensive collections of *Mytilus* from various localities around the Irish coasts, a total of 43 sites in all. He found the *M. galloprovincialis* form intermixed with *M. edulis* in varying proportions on the Atlantic coasts of Ireland but found no evidence for the occurrence of *M. galloprovincialis* along the east coast (Irish Sea Coast). At all Atlantic sites there was a considerable degree of overlap in morphological characters and the greatest number of intermediate forms were encountered on exposed shores.

A preliminary report (Gosling & Wilkins, 1977) on allele frequency at the *Pgi* locus in populations of *Mytilus* (approximately twenty sites) on Irish coasts indicated that mussels on the Irish Sea coast differed significantly in allele frequency from Atlantic exposed and sheltered shore mussels. Subsequent investigations using the *Lap-2* and *Pgm* loci supported this observation (Gosling & Wilkins, 1981). Mussels on the Irish Sea coast exhibited allele frequencies at the three loci which were very similar to populations of 'pure' *M. edulis* from the coast of France (Gosling & Wilkins, 1977, 1981) and Britain (Ahmad *et al.*, 1977). Allele frequencies were homogeneous over localities; there were no significant deviations from Hardy-Weinberg expectations at the three loci and the evidence suggested that mussels in this area constituted a single panmictic population of *M. edulis* alone. On the Atlantic coasts of Ireland there were marked differences in allele frequency at all loci between exposed and sheltered shore mussels and large significant deficiencies of heterozygotes at the *Pgi* locus were observed at all but one of the exposed sites while the majority of the sheltered shore samples did not exhibit such deficiencies. Heterozygote deficiencies were not correlated with absolute or relative size of individual mussel nor with position of the mussels on the shore. The indications were—from analyses of *M. galloprovincialis* from the Mediterranean and Northern France (Gosling & Wilkins 1977, 1981)—that the exposed shore samples, and to a much lesser extent the sheltered shore samples constituted a mixture of interbreeding *M. galloprovincialis* and *M. edulis* (Table 5).

No attempt was made to separate the two forms of mussel on exposed shores. However, at Lough Ine, a sheltered site on the S coast of Ireland, where previous workers (Baird, cited by Hepper, 1957; Kitching *et al.*, 1959; Seed, 1974) have reported a high incidence (58–77%) of the *M. galloprovincialis* form, mussels exhibited allele frequencies at the three loci which were very similar to exposed shore frequencies. Subsequently *M. edulis*-like individuals (approximately 33%) were separated from the sample but no statistical differences in allele frequency were observed at any of the three loci between these individuals and the remainder of the sample. Interestingly, the *M. edulis* group, in contrast to the total sample, did not exhibit significant deficiencies of heterozygotes at the *Pgi* locus relative to Hardy-Weinberg expectations.

It would appear therefore that in Ireland, at least, the *M. galloprovincialis* form occurs mainly at exposed sites, whereas *M. edulis* is more common in sheltered shore environments. Skibinski *et al.* (1983), using *Est-D* allele frequency data, have confirmed our

TABLE 5. Mean frequency of the major *Pgi, Lap-2* and *Pgm* alleles in pure *M. edulis* from San Vaast, N France, in Irish coastal mussels and in pure *M. galloprovincialis* from two sites in the Mediterranean (Cannes and Venice). Data from Gosling & Wilkins (1981). Allele numbers correspond to those in Ahmad & Beardmore (1976).

	Pgi					*Lap-2*				*Pgm*			
	N	1	3	4	5	N	2	3	4	N	2	3	4
M. edulis France	92	0.04	0.26	0.0	0.64	102	0.14	0.66	0.20	104	0.12	0.66	0.22
Mytilus Irish Sea	527	0.05	0.30	0.0	0.61	457	0.11	0.61	0.26	527	0.13	0.64	0.21
Atlantic sheltered	2219	0.05	0.37	0.02	0.52	2078	0.10	0.57	0.30	2137	0.13	0.60	0.25
Atlantic exposed	1138	0.04	0.44	0.15	0.29	1138	0.09	0.48	0.41	1138	0.14	0.57	0.28
M. gall. Mediterranean	154	0.01	0.83	0.13	0.02	149	0.05	0.52	0.43	68	0.10	0.53	0.34

observation. Unfortunately, a similar analysis has not been possible for British sites due to insufficient information on the degree of exposure of the sites investigated there. However, as Skibinski *et al.* (1983) point out "exposure score alone cannot be used to predict allele frequency" since at several sheltered sites on the Atlantic coasts of Ireland (Gosling & Wilkins, 1981) allele frequency and genotype proportions resembled those observed for exposed shore mussels. Also, mussels at the single exposed site sampled on the Irish Sea coast (Gosling & Wilkins, 1981) did not differ appreciably in allele frequency from the sheltered shore samples in the same area and did not exhibit deficiencies of heterozygotes at the *Pgi* locus. Skibinski *et al.* (1983) have also observed low frequencies of *Est-D*^{2} (a diagnostic allele for *M. galloprovincialis*) at moderately exposed sites on the Irish Sea coast of Ireland.

The results for Irish sites are interesting in the light of an earlier report by Murdock *et al.* (1975). These authors observed a significant correlation between frequencies of the common alleles at a *Lap* locus and exposure in samples of *Mytilus* from Irish coasts (It is not clear whether the *Lap* locus studied by Murdock *et al.* (1975) is *Lap-1*; examination of their allele frequency data suggest that it is not). This paper has been much cited as evidence for selection in mussel populations. The correlation between allele frequency and exposure may not in fact be a correlation with exposure *per se* but may reflect the higher incidence of *M. galloprovincialis* alleles at the more exposed sites investigated by Murdock *et al.* (1975).

The present distribution of *M. galloprovincialis* in W Europe may be inferred by using allele frequency data from the three best discriminatory loci. Fig. 2 illustrates that *M. galloprovincialis* is present on the SW peninsula of Britain and at a single location on the S coast of Wales (Skibinski & Beardmore, 1979); on the S, W and N coasts of Ireland (Gosling & Wilkins, 1977, 1981; Skibinski & Beardmore, 1979) on the NE coast of England, on the NW coast of Scotland (Skibinski & Beardmore, 1979) and as far N (60° N) as the Shetland and Orkney islands (Gosling, unpublished results). The distribution of *M. galloprovincialis* has been both confirmed and extended using electrophoretic data, with one exception: Skibinski & Beardmore (1979) found no evidence of *M. galloprovincialis* on the S coast of Wales outside of the King's Dock site. However, Hepper (and others cited by him) in 1957, using general shell shape and mantle edge colour as morphological criteria, found *M. galloprovincialis* at several locations on the S coast and at places, e.g. Cardiff; the proportion of *M. galloprovincialis* recorded was 60%. Either the morphological characters used by Hepper are worthless (and this may be true) or else both have sampled different sites on the S Wales coast. It is difficult to check this since location names have not been given for the sites sampled by Skibinski & Beardmore (1979).

The electrophoretic evidence suggests that where *M. galloprovincialis* and *M. edulis* are

FIG. 2. Map showing in heavy outline the geographical areas in which the *M. galloprovincialis* form has been detected using allele frequency data from the partially diagnostic loci *Pgi, Est-D* and *Lap-1*.

found together they are at least interbreeding and in some areas intergradation between the two forms is extensive. The extent of intergradation in mixed samples of mussels from British (16 sites) and Irish (9 sites) has been assessed by measuring deviations from Hardy-Weinberg expectations and genotypic correlations between *Est-D* and *Lap-1*. Three statistics have been used: F (Wright, 1951), which measures deviations from Hardy-Weinberg equilibrium, R1 which measures the strength of association of homozygote genotypes and R2 which measures the excess of double heterozygotes expected. General trends in the values of these statistics occur as a result of interbreeding: on hybridization F decreases, R2 increases and R1 remains unaltered and if intergradation proceeds, all three measures decrease at different rates to zero when the process is complete (Skibinski & Beardmore, 1979). In SW England hybridization but little intergradation is occurring between the two forms of mussel; at other localities, e.g. on the S coast of Ireland, intergradation, though not complete, is considerable. In general, the extent of intergradation is greater in Ireland, Scotland and NE England than in SW England.

In summary, electrophoretic analysis of allopatric populations of *M. edulis* and *M. galloprovincialis* have shown that the two forms are closely related; genetic identity and genetic distance values for comparisons between the two mussel types are similar to the mean values observed by various workers for comparisons between subspecies of other invertebrate taxa. In areas where the two forms are found together, e.g. the coasts of Britain and Ireland, electrophoretic investigations have enabled us to quantify the extent of hybridization and intergradation taking place between *M. edulis* and *M. galloprovincialis* in these areas. In addition, we now have more precise information on the micro- and macrogeographic distribution of the two forms in W Europe. However, while these results are interesting in themselves, they do not resolve the problem: is the *M. galloprovincialis* form a distinct species of *Mytilus* or is it merely a race or ecotype of its progenitor *M. edulis*?

Nearly all the most illuminating investigations of speciation have been those where a number of techniques have been employed. With this in mind, information from cytological, immunological and artificial hybridization studies is presented and discussed in the following section in an attempt to explore further the systematic relationship between the *M. edulis* and *M. galloprovincialis* forms.

Cytological Studies

The study of karyotypes has been used as a valuable complement to biochemical methods of identifying species, hybrids and more rarely, populations (Chevassus *et al.*, 1978). Karyotypes have been described for *M. edulis* (Ahmad & Sparks, 1970; Thiriot-Quiévreux & Ayraud, 1982; Moynihan & Mahon, 1983; Thiriot, this volume) and *M. galloprovincialis* (Thiriot-Quiévreux & Ayraud, 1982; Thiriot, this volume). While these authors agree that the two forms exhibit a diploid chromosome number of 28, their reports are at variance as to the karyotype of these two mussels. Ahmad & Sparks (1970), examining *M. edulis* from the W coast of North America, observed three pairs of acrocentric and a variable number of metacentric and submetacentric chromosome pairs. They also

observed structural chromosomal polymorphism within populations of *M. edulis* (and *M. californianus* Conrad) which they attributed to the presence of pericentric inversions or centromere shifts in the autosomes. Moynihan & Mahon (1983) observed 5–6 metacentric pairs, 6–8 submetacentric pairs and 0–3 subtelocentric pairs in *M. edulis* from Galway, on the W coast of Ireland (there was no evidence of the *M. galloprovincialis* form at this site [Gosling & Wilkins, 1981]). Moynihan & Mahon (1983) attributed the variation in the number of chromosome types to differential contraction of chromosomes rather than to any structural changes which could affect centromere position, e.g., pericentric inversions or non-reciprocal translocations. Thiriot-Quiévreux & Ayraud (1982) and Thiriot (this volume) have observed 2 pairs of metacentric, 6 pairs of submetacentric and 6 telocentric pairs in French populations of *M. edulis* from Charron ('pure' *M. edulis*) and Brest (mixture of *M. edulis* and *M. galloprovincialis*). Three populations of *M. galloprovincialis* were also analysed from Banyuls and Villefranche ('pure') and Brest (mixed). *M. edulis* from 'pure' and mixed populations differed consistently from *M. galloprovincialis* in that chromosome pair number 2 was metacentric in *M. edulis* but telocentric in *M. galloprovincialis*. This difference was ascribed to a pericentric inversion. Thiriot-Quiévreux & Ayraud (1982) concluded that the difference in the position of the centromere between *M. edulis* and *M. galloprovincialis* was further evidence that these two forms should be regarded as separate species.

Since certain types of chromosomal rearrangements, e.g. inversions and translocations, of various kinds may play a determining role in the speciation process (but see Zouros, 1982) in many groups of animals has been repeatedly suggested by White (1968, 1978). It is believed that such inversions could provide the raw material for the forces of speciation which would eventually convert these polymorphisms within the ancestral population into a system of fixed inversions in two lineages. While this may be an attractive hypothesis to explain the chromosomal differences observed between the two mussel types it must be treated with caution for the following reasons:

1. There appear to be considerable technical difficulties with karyotypic analysis in *Mytilus,* as evidenced by the lack of congruence between the results obtained by various investigators.
2. To date, very few allopatric and sympatric populations of *M. edulis* and *M. galloprovincialis* have been analysed cytologically.
3. Polymorphisms for pericentric inversions have been observed within and between populations of *M. edulis* itself (Ahmad & Sparks, 1970).

Karyotypic changes are *not* universal for speciation; for example, homosequential species complexes have been observed in *Drosophila (D. mulleri, D. aldrichi* and *D. wheeleri*). On the other hand, certain ecological preferences can be tied up with chromosomal polymorphism within a single species, e.g. the marine snail *Nucella lapillus,* where a polymorphism for chromosome number has been observed (Staiger, 1955). Populations on exposed shores exhibit a diploid chromosome number of 26 while those on sheltered shores have 36 chromosomes. Heterogeneous colonies with intermediate chromosome frequencies (26–36) have been observed in intermediate localities.

In conclusion, there is no valid reason, on the basis of a single pericentric inversion, to consider *M. galloprovincialis* as a distinct species of *Mytilus.* Further work on allopatric and sympatric populations of the two forms is in progress (C. Thiriot and D. Dixon, personal communications, 1982). In addition, the karyotypic analysis of wild, e.g. exposed shore populations, and artificially-produced 'hybrids' between *M. galloprovincialis* and *M. edulis* is also underway (C. Thiriot and P. Lubet, personal communications, 1982).

Immunological Studies

Serological methods are relatively free of environmentally-induced changes, being based on the quantitative precipitation of proteins. Moreover, when the precipitations are performed in gel-immunodiffusion or immunoelectrophoresis the sensitivity of serological methods is enhanced and even the rate of cross-reactivity between individuals very close on the zoological scale can be appreciated. Such an approach has been used by Bisignano *et al.* (1980) to compare the muscle protein antigens extracted from samples of *M. galloprovincialis* (Sicily) and *M. edulis* (location not stated). These authors observed that all of the antigens present in *M. galloprovincialis* were present also in *M. edulis* and that *M.*

edulis possessed one extra antigen not observed in *M. galloprovincialis*. Also, one antigen present in *M. edulis* was present only at low concentrations in *M. galloprovincialis*. On the basis of these results the authors concluded that *M. galloprovincialis* was indeed a distinct species derived from *M. edulis*.

Since there is little published information on immunological comparisons between conspecific populations, congeneric species or species of different genera of molluscs it is difficult to evaluate the results of Bisignano *et al.* (1980). Two reports, however, are worth discussing. Investigating the genetic similarities between the closely related cockle species *Cardium edule* and *C. glaucum* using crossed immunoelectrophoresis, Brock (1980) observed seven species-specific antigen-antibody reactions between the two species. It is unlikely that these reflect intraspecific variation, as the same compatibility/non-compatibility patterns were observed for different populations of the two species, both allopatric and sympatric. The high compatibility between antigens of *C. edule* and *C. glaucum* led Brock to conclude that these are closely related; the discovery of seven species-specific antigens indicated that they should, however, be regarded as distinct species. This agrees with ecological (Brock, 1979) and genetic (Gosling, 1980) evidence to date. Additional investigations on a species of the same genus, *C. echinatum* (believed by some authorities [Bowden & Heppell, 1966; Fischer-Piette, 1977] to belong to the genus *Acanthocardia*), showed 17 and 19 species-specific antigens for the *C. echinatum/C. glaucum* and *C. echinatum/C. edule* comparisons respectively.

Davis & Fuller (1981) have carried out an impressive immunological survey on 52 species belonging to 27 genera of North American Unionacea, the object being to establish a classification based on the immunology, morphology, fossil record and zoogeograpy of this large group of freshwater bivalves. Cross comparisons between species pairs of the same genus (*Elliptio, Anodonta, Fusconaia, Margaritifera, Quadrula* and *Lampsilis*) indicated that they differed by one to six antigens with most species pairs differing by more than two antigens. When species of different genera were compared the average antigen difference between them was approximately six. These values are lower than those observed by Brock for species of *Cardium*. This is not unexpected since the relationship between immunoelectrophoretic genetic distance and taxonomic hierarchy—presumably based on comparative morphology—is not a simple one. There is in fact no direct correspondence (Davis & Fuller, 1981) except in cases where protein and organismic evolution both have proceeded in a regular fashion with regard to elapsed time (Ferguson, 1980). This further emphasizes the desirability of applying a multi-pronged approach to taxonomic problems.

In light of the above reports, what inferences can be drawn from the results of Bisignano *et al.* (1980) for the two forms of *Mytilus*? On the basis of one antigen difference they could be regarded as separate species (Davis & Fuller, 1981). On the other hand, the differences observed by Bisignano *et al.* (1980) could be simply intraspecific variation since, apparently, only one population of *M. edulis* was compared with one of *M. galloprovincialis*. Further work is necessary in order to determine whether immunological differences persist when allopatric and sympatric populations of the two forms are analysed. Brock (personal communication, 1982) used crossed immunoelectrophoresis but was unable to distinguish two types of mussels in mixed samples from Irish coasts. Immunological investigations on sympatric populations of the two forms of mussels from areas such as SW England, where morphological, genetic and physiological differences are more pronounced, could be informative. But until further work is carried out there is little to conclude regarding the systematic status of the *M. galloprovincialis* from the immunological evidence to date.

Artificial hybridization

There has been only one report to date on artificial hybridization between *M. edulis* and *M. galloprovincialis* (Lubet *et al.*, 1984) Reciprocal crosses have been carried out between *M. edulis* from Luc-sur-Mer, Normandy, France and *M. galloprovincialis* from Vigo, Spain. The control crosses within each mussel type were also accomplished. The F_1 larvae of all crosses were reared under laboratory conditions for a period of one year and then transferred to the wild for two years. During this time all individuals were reared under identical conditions of temperature, salinity, nutrition, population density, etc. While larval mortality was not estimated in the

different crosses, other parameters, i.e., incidence of larval abnormalities, time taken to reach metamorphosis, and juvenile (up to 10 mm) and adult (up to 55 mm) mortalities, were recorded. No significant differences were observed between the different crosses. Analysis of the relative growth rates (height/length of shell) for each cross showed no significant difference between 'hybrid' and 'pure' offspring; there was no evidence of heterosis for any of the characters measured. Backcrosses of 'hybrid' F_1 individuals to *M. edulis* F_1 and *M. galloprovincialis* F_1 were also performed and the same techniques were employed in the rearing of the F_2 generations. Unfortunately, all of the F_2 generations, having reached a length of approximately one cm, were lost in a storm. We now know, however, that when *M. galloprovincialis* and *M. edulis* are crossed they can produce fertile 'hybrids' and that these can backcross to the parent forms to produce viable (and perhaps fertile) offspring. Therefore, there is little evidence of genetic incompatibility between the two forms from laboratory-based studies. These results of Lubet *et al.* (1984) support the field evidence, based on morphological and electrophoretic data, of widespread hybridization and introgression between the two forms of mussel in W Europe.

DISCUSSION AND CONCLUSIONS

Morphological, electrophoretic, cytological and immunological studies on the two forms of mussel *M. edulis* and *M. galloprovincialis* indicate that they are very similar to one another with no *single,* morphological or genetic character being clearly diagnostic. This close similarity would not be sufficient grounds, however, for considering the two forms as varieties of a single species since there are several examples in the literature (Gottlieb & Pilz, 1976; Rick *et al.,* 1976; Crawford & Smith, 1982; Harrison & Arnold, 1982) of closely related but distinct species pairs of plants and animals that bear a close morphological and genetic similarity to one another. In almost all such cases, however, there are barriers to gene exchange between the species concerned. For example, in the very closely related cricket species *Gryllus pennsylvanicus* and *G. firmus* (Harrison & Arnold, 1982) laboratory crosses of *G. firmus* males and *G. pennsylvanicus* females produced apparently normal F_1 hybrids and from these F_2 and backcross individuals. The reciprocal cross, however, has consistently failed to produce offspring in the laboratory, indicating that a partial postmating barrier exists between the two species. In the case of the two closely-related plant species *Coreopsis nuecensoides* and *C. nuecensis* (Crawford & Smith, 1982) interspecific hybridization produces vigorous but completely sterile F_1 plants.

Evidence from morphological (Seed, 1978) and electrophoretic (Gosling & Wilkins, 1981; Skibinski & Beardmore, 1979) analyses has indicated that where *M. edulis* and *M. galloprovincialis* are found together they hybridize. (In the *M. edulis*/*M. galloprovincialis* case the use of the term 'hybridization' is no doubt erroneous (see Mayr, 1963) but is used for the sake of simplicity.) In addition, introgression is widespread especially in parts of Scotland, NE England and at exposed locations on the Atlantic coasts of Ireland. Of course one of the most difficult problems in biology is deciding how much interbreeding can take place before two populations are considered one rather than two species. The evidence so far suggests that the amount of genetic mixing between the two forms "is such as to suggest strongly that there is no isolating barrier between the two types of mussel . . . even in SW England" (where the amount of interbreeding is less); "there is no clear genetic evidence of an absolute reproductive barrier between the types and thus *no good reason to regard them as distinct species* [my italics]" (Skibinski *et al.,* 1983). This conclusion is supported by the results from artificial hybridization studies (Lubet *et al.,* 1984) which indicate that there is little evidence of genetic incompatibility between the two forms. The two types cross readily with each other to produce fertile F_1 individuals and there are no indications of reduced viability or growth rate or increased mortality among the F_1 'hybrids' compared to the parental crosses.

Whether the two forms are in the process of differentiation (incipient sympatric speciation) or whether they are in the process of integrading after a period of allopatry is impossible to determine. The allopatric model is supported by Barsotti & Meluzzi (1968) who believe that *M. galloprovincialis* is a recent derivative of *M. edulis*, having arisen allopatrically in the Mediterranean during a Pleistocene ice age. The present-day distribution of the *M. galloprovincialis* form in W Europe represents a

northward expansion of its range since the Pleistocene era. Skibinski *et al.* (1983) suggest that the similarity both in morphological and allozymic characteristics between British and Mediterranean *M. galloprovincialis* are so great as to provide strong support for the allopatry model; there is little supporting evidence for the sympatry model, especially since so few clear examples of sympatric speciation have been described in the literature to date (for a full discussion on this topic see Skibinski *et al.*, 1983). Whether the *M. galloprovincialis* form has arisen in sympatry or in allopatry does not alter the conclusions of this paper, i.e. that the *M. galloprovincialis* form does not merit the rank of full species, but appears, from the evidence to date, to be a variety or ecotype of the larger *Mytilus edulis* species complex.

Accepting that the two mussel types have secondarily come in contact and are freely hybridizing, there are two possible outcomes: either complete intergradation leading to eventual fusion of the two partially differentiated gene pools or, if selection is operating against hybrids, further differentiation and the reinforcement of reproductive isolating barriers. To determine which of these processes is actually occurring would require detailed ecological and genetic analyses of mussel populations of mixed ancestry from selected sites in Britain and Ireland. (For a full discussion on the characteristics and dynamics of hybrid zones the reader is referred to: Mayr, 1963; Lewontin & Birch, 1966; Remington, 1968; Littlejohn *et al.*, 1971; Watson *et al.*, 1971; Watson, 1972; Hunt & Selander, 1973; Moore, 1977; Blackwell & Bull, 1978; Skibinski *et al.*, 1978a, b; Barton, 1979; Gartside *et al.*, 1979; Skibinski & Beardmore, 1979; Woodruff & Gould, 1980; Barton, 1981; Skibinski *et al.* 1983.) Sites such as Rock and Croyde in SW England, where there is little intergradation occurring between the two mussle types, and sites such as exposed locations on the W coast of Ireland, where there is almost complete intergradation, could be surveyed in depth with a view to answering the following questions:

In SW England:

a) Are there barriers to gene exchange between *M. edulis* and *M. galloprovincialis*, i.e. do they have different spawning cyles? Seed (1971) demonstrated differences in the reproductive cycles of the two forms at Rock; here *M. edulis* spawned about eight weeks earlier (May–June) than *M. galloprovincialis* (July–August). It would be interesting to know whether these differences are evident at other sites in the area.
b) Do 'hybrids' have lower fitness than the two parental forms? If chromosomal differences are shown to exist between the two types of mussels in this area then there could be selection against unbalanced heterozygous chromosomal arrangements in the 'hybrids.' Is there selection against introgressed genes? Is there differential introgression of allozyme alleles into the parental gene pools? The use of allozyme markers to measure the extent of introgression between closely related species has been well documented (Hunt & Selander, 1973; Avise & Smith, 1974; Gartside *et al.*, 1979; Skibinski & Beardmore, 1979).
c) Accepting that the two forms of mussels are genetically different, is the extent of differentiation between them increasing or decreasing with time? To answer this question long-term electrophoretic analyses of 'hybrid' populations would be necessary. There may of course be two processes occurring in such populations, as Skibinski *et al.*, 1983 suggest: "the homogenizing effect of interbreeding and the differentiating effect of selection might balance one another resulting in temporal stability of population genetic structure from year to year."

In the case of exposed shore populations on the coasts of Ireland some of the questions might be:

a) Why does the *M. galloprovincialis* form favour the exposed shore environment?
b) Is there evidence of microgeographic separation of the two forms in this habitat, i.e. does the *M. galloprovincialis* form favour the upper shore as opposed to the lower shore?
c) Why is there little or no evidence for the *M. galloprovincialis* form on sheltered Atlantic shores in Ireland, in spite of the long pelagic life (4–6 weeks) of *Mytilus* larvae? Do *M. galloprovincialis* larvae settle but not survive because of competition from *M. edulis* at sheltered sites? This question is probably impossible to answer since the majority of the *M. galloprovincialis* form may die before reaching a size large enough to be sampled for electrophoresis. It must be remembered of course that at some sheltered locations, e.g. Lough Hyne on the

south coast of Ireland (Gosling & Wilkins, 1981) the *M. galloprovincialis* form is abundant and thriving. Why this can occur at some sheltered sites and not at others is not understood.

d) Is the process of intergradation between the two forms on exposed shores (and at other locations, e.g. NE England, parts of Scotland) increasing with time? It is possible that complete fusion of the two gene pools may be in progress and may ultimately produce an ecotype that is particularly well-suited to the rigorous environment of the exposed shore.

In conclusion, the evidence from morphological, electrophoretic, cytological, immunological and artificial hybridization studies now indicate that *M. galloprovincialis* is closely related to *M. edulis* but does not merit the rank of full species. It would appear from the evidence to date that *M. galloprovincialis* is a variety, ecotype or perhaps subspecies of the larger *M. edulis* species complex. The *exact* status of *M. galloprovincialis* still remains an intriguing taxonomic problem, one which will probably occupy the efforts of many investigators in the years to come. In the meantime, it is proposed that the name *Mytilus edulis* var. *galloprovincialis* be used to describe the *galloprovincialis* form of mussel, following Jeffreys (1863).

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REFERENCES CITED

AHMAD, M. & BEARDMORE, J. A., 1976, Genetic evidence that the "Padstow Mussel" is *Mytilus galloprovincialis*. *Marine Biology*, 35: 139–147.

AHMAD, M., SKIBINSKI, D. O. F. & BEARDMORE, J. A., 1977, An estimate of the amount of genetic variation in the common mussel *Mytilus edulis*. *Biochemical Genetics*, 15: 833–846.

AHMAD, M. & SPARKS, A. K., 1970, Chromosome number, structure and autosomal polymorphism in the marine mussels *Mytilus edulis* and *Mytilus californianus*. *Biological Bulletin*, 138: 1–13.

AVISE, J. C., 1974, Systematic value of electrophoretic data. *Systematic Zoology*, 23: 465–481.

AVISE, J. C., 1976, Genetic differentiation during speciation. In: AYALA, F.J., ed., *Molecular Evolution*, Sinauer Associates, Sunderland, Massachusetts, p. 106–122.

AVISE, J. C. & SMITH, M. H., 1974, Biochemical genetics of sunfish. I. Geographic variation and subspecific intergradation in the bluegill, *Lepomis macrochirus*. *Evolution*, 28: 42–56.

AYALA, F. J., TRACEY, M. L., BARR, L. G., McDONALD, J. F. & PÉREZ-SALAS, S., 1974, Genetic variation in natural populations of five *Drosophila* species and the hypothesis of the selective neutrality of protein polymorphisms. *Genetics*, 77: 343–384.

BARSOTTI, G. & MELUZZI, C., 1968, Osservazioni su *Mytilus edulis* L. e *Mytilus galloprovincialis* Lamarck. *Conchiglie*, 4: 50–58.

BARTON, N. H., 1979, The dynamics of hybrid zones. *Heredity*, 43: 341–359.

BARTON, N. H., 1981, THe width of the hybrid zone. *Heredity*, 47: 283–292.

BISIGNANO, G., DI PIETRO, A., GRILLO, O. C. & PICERNO, I., 1980, Serological studies on the genus *Mytilus*. Comparison of proteinic antigens extracted from *M. edulis* and *M. galloprovincialis*. *Revue International d'Océanographie Medicale*, 59: 31–39.

BLACKWELL, J. M. & BULL, C. M., 1978, A narrow hybrid zone between two western Australian frog species *Ranidella insignifera* and *R. pseudinsignifera*: the extent of introgression. *Heredity*, 40: 13–25.

BOWDEN, J. & HEPPELL, D., 1966, Revised list of British Mollusca. 2. Unionacea-Cardiacea. *Journal of Conchology*, 26: 237–272.

BROCK, V., 1979, Habitat selection of two congeneric bivalves, *Cardium edule* and *C. glaucum* in sympatric and allopatric populations. *Marine Biology*, 54: 149–156.

BROCK, V., 1980, Ph.D. thesis. University of Aarhus, Denmark.

CHEVASSUS, B., BLANC, J. M. & GUYOMARD, R., 1978, Les recherches en génétique appliquée à l'aquiculture. *Bulletin Scientifique et Technique*. Departement d'Hydrobiologie, I.N.R.A. No. 3.

CRAWFORD, D. J. & SMITH, E. B., 1982, Allozyme variation in *Coreopsis nuecensoides* and *C. nuecensis* (Compositae), a progenitor-derivative species pair. *Evolution*, 36: 379–386.

DAVIS, G. M. & FULLER, S. L. H., 1981, Genetic relationships among recent Unionacea (Bivalvia) of North America. *Malacologia*, 20: 217–253.

DAVIS, G. M., HEARD, W., FULLER, S. L. H. & HESTERMAN, C., 1981, Molecular genetics and speciation in *Elliptio*, and its relationship to other taxa of North American Unionidae (Bivalvia). *Biological Journal of the Linnean Society*, 15: 131–150.

FERGUSON, A., 1980, *Biochemical Systematics and Evolution*. Blackie, Glasgow and London, 194 p.

FISCHER-PIETTE, E., 1977, Révision des Cardiidae (Mollusques: Lamellibranches). *Mémoires du Museum National d'Histoire Naturelle,* 101: 212 p.

GARTSIDE, D. F., LITTLEJOHN, M. J. & WATSON, G. F., 1979, Structure and dynamics of a narrow hybrid zone between *Geocrinia laevis* and *G. victoriana* (Anura: Leptodactylidae) in south-eastern Australia. *Heredity,* 43: 165–177.

GOSLING, E. M., 1980, Gene frequency changes and adaptation in marine cockles. *Nature,* 286: 601–602.

GOSLING, E. M. & WILKINS, N. P., 1977, Phosphoglucosisomerase allele frequency data in *Mytilus edulis* from Irish coastal sites: its ecological significance. In: KEEGAN, B. F., Ó CÉIDIGH, P. & BOADEN, P. S., eds., *Biology of Benthic Organisms,* Pergamon, London, p. 297–309.

GOSLING, E. M. & WILKINS, N. P., 1981, Ecological genetics of the mussels *Mutilus edulis* and *M. galloprovincialis* on Irish coasts. *Marine Ecology Progress Series,* 4: 221–227.

GOTTLIEB, L. D. & PILZ, G., 1976, Genetic similarity between *Gaura longiflora* and its obligately outcrossing derivative *G. demareei. Systematic Botany,* 1: 181–187.

GOULD, S. J. & WOODRUFF, D. S., 1978, Natural history of *Cerion.* VIII: Little Bahama Bank—A revision based on genetics, morphometrics, and geographic distribution. *Bulletin of the Museum of Comparative Zoology,* 148: 371–415.

HARRISON, R. G. & ARNOLD, J., 1982, A narrow hybrid zone between closely related cricket species. *Evolution,* 36: 535–552.

HEPPER, B. T., 1957, Notes on *Mytilus galloprovincialis* (Lmk.) in Great Britain. *Journal of the Marine Biological Association of the United Kingdom,* 36: 33–40.

HUNT, W. G. & SELANDER, R. K., 1973, Biochemical genetics of hybridization in European house mice (*Mus musculus*). *Heredity,* 31: 11–33.

JEFFREYS, J. G., 1863, *British Conchology,* Van Voorst, London, 2: 105.

JOHNSON, M. S., CLARKE, B. & MURRAY, J., 1977, Genetic variation and reproductive isolation in *Partula. Evolution,* 31: 116–126.

KITCHING, J. A., SLOANE, J. F. & EBLING, F. J., 1959, The ecology of Lough Ine. VIII. Mussels and their predators. *Journal of Animal Ecology,* 28: 331–341.

LEWIS, J. R. & POWELL, H. T., 1961, The occurrence of curved and ungulate forms of the mussel *Mytilus edulis* (L.) in the British Isles, and their relationship to *M. galloprovincialis* (Lmk.). *Proceedings of the Zoological Society of London,* 137: 583–598.

LEWIS, J. R. & SEED, R., 1969, Morphological variations in *Mytilus* from S.W. England in relation to the occurrence of *Mytilus galloprovincialis* (Lmk.). *Cahiers de Biologie Marine,* 10: 231–253.

LEWONTIN, R. C. & BIRCH, L. C., 1966, Hybridization as a source of variation for adaptation to new environments. *Evolution,* 20: 315–336.

LITTLEJOHN, M. J., WATSON, G. F. & LOFTUS-HILLS, J. J., 1971, Contact hybridization in the *Crinia laevis* complex (Anura: Leptodactylidae) *Australian Journal of Zoology,* 19: 85–100

LUBET, P., 1959, Recherches sur le cycle sexuel et l'emission des gametes chez les Mytilidés et les Pectinidés. *Revue des travaux de l'Office Scientifique et Technologique des Pêches maritimes,* 23: 387–548.

LUBET, P., 1973, Exposé synoptique des données biologiques sur la moule *Mytilus galloprovincialis* (Lmk.). *Synopsis FAO sur les Pêches,* 88: 1–8.

LUBET, P., MASSON, M., PRUNUS, G. & BUCAILLE, D., 1984, Etude expérimentale du croisement *Mytilus edulis* L. × *M. galloprovincialis* Lmk. *Bulletin de la Societé Zoologique de France.*

MAYR, E., 1963, *Animal Species and Evolution.* Harvard University Press, Cambridge, Massachusetts, 796 p.

MOORE, W. S., 1977, An evaluation of narrow hybrid zones in vertebrates. *Quarterly Review of Biology,* 52: 263–277.

MOYNIHAN, E. P. & MAHON, G. A. T., 1983, Quantitative kar[y]otype analysis in the mussel *Mytilus edulis* L. *Aquaculture,* 33: 301–309.

MURDOCK, E. A., FERGUSON, A. & SEED, R., 1975, Geographical variation in leucine aminopeptidase in *Mytilus edulis* L. from the Irish coasts. *Journal of Experimental Marine Biology and Ecology,* 19: 33–41.

NEI, M., 1972, Genetic distance between populations. *American Naturalist,* 106: 283–292.

REMINGTON, C. L., 1968, Suture zones of hybrid interaction between recently joined biotas. *Evolutionary Biology,* 2: 321–428.

RICK, C. M., KESICKI, E., FOBES, J. F. & HOLLE, M., 1976, Genetic and biosystematic studies on two new sibling species of *Lycopersicon* from interandean Peru. *Theoretical and Applied Genetics,* 47: 55–68.

SARICH, V. M., 1977, Rates, sample sizes and the neutrality hypothesis for electrophoresis in evolutionary studies. *Nature,* 265: 24–28.

SEED, R., 1968, Factors influencing shell shape in *Mytilus edulis* (L.) *Journal of the Marine Biological Association of the United Kingdom,* 48: 561–584.

SEED, R., 1971, A physiological and biochemical approach to the taxonomy of *Mytilus edulis* (L.) and *M. galloprovincialis* (Lmk.) from S.W. England. *Cahiers de Biologie Marine,* 12: 291–322.

SEED, R., 1972, Morphological variations in *Mytilus* from the French coasts in relation to the occurrence and distribution of *Mytilus galloprovincialis* (Lmk.). *Cahiers de Biologie Marine,* 13: 357–384.

SEED, R., 1974, Morphological variations in *Mytilus* from the Irish coasts in relation to the occurrence and distribution of *Mytilus galloprovincialis* (Lmk.). *Cahiers de Biologie Marine,* 15: 1–25.

SEED, R., 1978, The systematics and evolution of

Mytilus galloprovincialis (Lmk.). In: BATTAGLIA, B. & BEARDMORE, J. A., eds., *Marine Organisms: Genetics, Ecology and Evolution.* Plenum Press, London, p. 447–468.

SENE, F. M. & CARSON, H. L., 1977, Genetic variation in Hawaiian *Drosophila.* IV. Allozymic similarity between *D. silvestris* and *D. heteroneura* from the island of Hawaii. *Genetics,* 86: 187–198.

SKIBINSKI, D. O. F. & BEARDMORE, J. A., 1979, A genetic study of intergradation between *Mytilus edulis* and *M. galloprovincialis. Experientia,* 35: 1442–1444.

SKIBINSKI, D. O. F., BEARDMORE, J. A. & AHMAD, M., 1978a, Genetic aids to the study of closely related taxa of the genus *Mytilus.* In: BATTAGLIA, B. & BEARDMORE, J. A., eds., *Marine Organisms: Genetics, Ecology and Evolution.* Plenum Press, London, p. 469–485.

SKIBINSKI, D. O. F., AHMAD, M. & BEARDMORE, J. A., 1978b, Genetic evidence for naturally occurring hybrids between *Mytilus edulis* and *Mytilus galloprovincialis. Evolution,* 32: 354–364.

SKIBINSKI, D. O. F., CROSS, T. F. & AHMAD, M., 1980, Electrophoretic investigation of systematic relationships in the marine mussels *Modiolus modiolus* L., *Mytilus edulis* L. and *Mytilus galloprovincialis* Lmk. (Mytilidae; Mollusca). *Biological Journal of the Linnean Society,* 13: 65–73.

SKIBINSKI, D. O. F., BEARDMORE, J. A. & CROSS, T. F., 1983, Aspects of the population genetics of *Mytilus* (Mytilidae; Mollusca) in the British Isles. *Biological Journal of the Linnean Society,* 19: 137–183.

SNYDER, T. P. & GOOCH, J. L., 1973, Genetic differentiation in *Littorina saxatilis* (Gastropoda). *Marine Biology,* 22: 177–182.

STAIGER, H., 1955, Reziproke Translokationen in natülichen Poulationen von *Purpura lapillus* (Prosobranchia). *Chromosoma,* 7: 181–197.

THEISEN, B. F., 1978, Allozyme clines and evidence of strong selection at three loci in *Mytilus edulis* L. (Bivalvia) from Danish waters. *Ophelia,* 17: 135–142.

THIRIOT-QUIÉVREUX, C., 1983, Les caryotypes de quelques Ostreidae et Mytilidae. *Malacologia,* 25: 465–476.

THIRIOT-QUIÉVREUX, C. & AYRAUD, N., 1982, Les caryotypes de quelques espèces de bivalves et de gastropodes marins. *Marine Biology,* 70: 165–172.

THORPE, J. P., BEARDMORE, J. A. & RYLAND, J. S., 1978, Taxonomy, interspecific variation and genetic distance in the phylum Bryozoa. In: BATTAGLIA, B. & BEARDMORE, J. A., eds., *Marine Organisms: Genetics, Ecology* and *Evolution.* Plenum, London, p. 425–445.

WATSON, G. F., 1972, The *Litoria ewingi* complex (Anura: Hylidae) in south-eastern Australia. II. Genetic incompatibility and delimitation of a narrow hybrid zone between *L. ewingi* and *L. paraewingi. Australian Journal of Zoology,* 20: 423–433.

WATSON, G. F., LOFTUS-HILLS, J. J. & LITTLEJOHN, M. J., 1971, The *Litoria ewingi* complex (Anura: Hylidae) in southeastern Australia. I. A new species from Victoria. *Australian Journal of Zoology,* 19: 401–416.

WHITE, M. J. D., 1968, Models of speciation. *Science,* 159: 1065–1070.

WHITE, M. J. D., 1978, *Modes of speciation.* Freeman, San Francisco, 455 p.

WOODRUFF, D. S. & GOULD, S. J., 1980, Geographic differentiation and speciation in *Cerion*—a preliminary discussion of patterns and processes. *Biological Journal of the Linnean Society,* 14: 389–416.

WRIGHT, S., 1951, The genetical structure of populations. *Annals of Eugenics,* 15: 323–354.

ZOUROS, E., 1982, On the role of chromosomal inversions in speciation. *Evolution,* 36: 414–416.

MALACOLOGIA, 1984, 25(2): 569–581

EXCESS OF ALLOZYME HOMOZYGOSITY IN MARINE MOLLUSCS AND ITS POSSIBLE BIOLOGICAL SIGNIFICANCE

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ABSTRACT

Several studies of electrophoretically detected enzyme variations have reported homozygote frequencies higher than expected under panmixia and random mating. Such excess of homozygosity has repeatedly been observed in over two dozen bivalves, including *Brachidontes, Crassostrea, Macoma, Modiolus* and *Mytilus*. Possible explanations for such an observation fall in four categories: inbreeding, presence of null alleles, Wahlund effect and selection. These species are in general dioecious with external fertilization, and therefore avoid inbreeding. An excess of homozygosity has been observed for several enzyme loci. The presence of many common null alleles on all these randomly selected polymorphic loci is not likely. Aspects of the Wahlund effect (a consequence of sampling over populations each of which behaves as a separate Mendelian population), and of selection are presented using data on *Crassostrea* and *Macoma* along with a summary of observations on *Mytilus*. The data on excess homozygosity presented here have three special features: (1) The degree of this excess is dependent on age and stage of development, higher in individuals of younger than of older age groups, (2) Degree of homozygosity has a negative correlation with growth rate and (3) Slow growers have a higher post-settlement mortality rate. Such results could not be explained by the Wahlund effect.

These observations permit us to offer a hypothesis for the origin and existence of excess homozygosity observed in these species with pelagic larvae. Depending on the species the pelagic larval period may range from three to four weeks after which they settle to form spat and grow to maturity. Furthermore, the time to first spawning tends to be a function of size rather than age, and individuals continue to reproduce after first spawning as long as they stay in the population. This model could be viewed as a form of balancing selection, where the relative fitness of homozygotes and heterozygotes is different during the pelagic larval phase from stages following settlement. Differential fitness based on development stages has been reported in other organisms and there is no reason why it should not be possible for molluscs.

Key words: enzyme polymorphism; excess of homozygosity; deficiency of heterozygotes; heterozygote superiority; growth rate; selective mortality; balancing selection.

INTRODUCTION

Larvae are the main instrument of dissemination for most sessile benthic invertebrate species. The planktotrophic pelagic larvae usually reach the stage and size of settlement within two to three weeks. Such species have had to evolve mechanisms to reaggregate in order to breed. Pelagic dispersal is also associated with high fecundity to offset the mortality experienced during the larva's sojourn in the sea (Crisp, 1977). Williams (1975) calls this situation the "Elm-Oyster Model" referring to the close analogy between larvae and wind-born seeds. At least in plants large genetic components of variability relating to features of biological significance (i.e. vigour, fertility, germination, tolerance and seedling mortality) have been demonstrated (Stebbins, 1970; Frip & Caten, 1971) and form the basis for plant breeding methodologies. The analogy between larvae and seeds is clearly of significance to the success of shellfish hatcheries. These features of marine molluscs in particular are also suitable for theoretical studies in evaluating fitness parameters of electrophoretic genotypes. In particular, being sessile in nature, they are exposed to local climatic conditions that can be followed with ease. Growth-rate is an important feature of biological significance for molluscs. Time to first spawning is dependent on size (not age) and determined by growth rate (Singh, 1978). Once they reach spawning size these individuals release gametes every spawning season while living in

the population. Also, the number of gametes released by a given individual in general is related to its size. Furthermore, fast growing individuals show lower mortality as compared to their slow growing counterparts (Haley & Newkirk, 1977). Growth rate is easily measured in molluscs by taking weight or other related measurements at timely intervals. Furthermore, experimental individuals could be grown under controlled conditions to minimize environmental differences during the growing period.

The biological features of molluscs are suitable for their use in genetical studies. Particularly, they have been used extensively in population genetic studies involving allozyme variations. Most such studies have shown homozygosity excess and heterozygote deficiency at a number of enzyme loci in over two dozen bivalve species (see Zouros & Foltz, 1984, for review). Possible explanations for such observations fall into four biological categories; each individually or in combination has been preferred by different authors. These include, presence of null alleles, allelozyme devoid of enzyme activity (Milkman & Beaty, 1970); inbreeding and self fertilization (Hornbach *et al.,* 1980); Wahlund effect, sampling over sub-population with different genetic composition each one of which behaves as a separate Mendelian population (Tracey *et al.,* 1975; Koehn *et al.,* 1976) and selection (Koehn & Mitton, 1972; Koehn *et al.,* 1973; Boyer, 1974; Gartner-Kepkay *et al.,* 1980). Ayala *et al.* (1973) and Buroker *et al.* (1975) also suggested misclassification of heterozygotes as homozygotes, due to poor electrophoretic resolution, as the technical possibility which may explain excess of homozygosity. In an earlier report we (Zouros *et al.,* 1980) have argued against inbreeding, null alleles and Wahlung effect as a possible cause of the observations on excess of homozygosity in molluscs in general and *Crassostrea virginica* in particular. Our data set (Singh & Zouros, 1978; Zouros *et al.,* 1980; Singh, 1982; this report) favours a form of balancing selection as an explanation for the origin and maintenance of homozygosity excess, which is based on developmental stage dependent heterozygote superiority. It is easy to evaluate the growth rate, age and electrophoretic genotype of molluscs from natural or experimental populations. Such a data set could be used to directly evaluate the relative fitness values for different genotypes through time during ontogeny of the individual and changes in populations. In this report we will argue for the selective nature of electrophoretic genotypes using data on three species of molluscs. The evidence for the selective or neutral nature of electrophoretic variation when gene frequencies are the main observable variables in laboratory and natural populations have been reviewed by Hedrick *et al.* (1976) and Nevo (1978).

1. *Mytilus* species

Mytilus is normally dioecious and post-larval individuals are sedentary. Adults may live up to six years, each releasing as many as 12 million gametes in a single spawn (Field, 1922). Zygotes that are produced in surrounding waters develop into mature larvae, ready for settling, following a number of ontogenetic stages (Bayne, 1965). Larvae stay in dispersal phase for over three weeks in high densities depending on tide, current and a number of other physical conditions.

Species of mussels are among the most commonly studied molluscs for electrophoretic genetic variations. These studies include a number of populations from wide geographic localities representing the east (e.g. Koehn *et al.,* 1976) and west (e.g. Tracey *et al.,* 1975) coasts of North America, Europe (e.g. Skibinski *et al.,* 1977, 1978) and Far East (e.g. Kartavtsev & Pudovkin, 1977). Most such studies have shown an excess of homozygosity at one or more enzyme loci. The species studied include: *Mytilus californianus* (Levinton & Fundiller, 1975; Tracey *et al.,* 1975), *M. edulis* (Milkman & Beaty, 1970; Koehn & Mitton, 1972; Koehn *et al.,* 1976; Johnson & Utter, 1973; Mitton *et al.,* 1973; Lassen & Turano, 1978; Gartner-Kepkay *et al.,* 1980), *M. galloprovincialis* (Skibinski *et al.,* 1977, 1978; Skibinski & Beardmore, 1979), *Modiolus auriculatus* (Lavee & Ritte, 1977), *M. demissus* (Koehn & Mitton, 1972; Koehn *et al.,* 1973) and *Crenomytilus grayanus* (Kartavtsev & Pudovkin, 1977), among others. In general the excess of homozygosity (or deficiency of heterozygotes) is evaluated by the use of *D* statistics, where D = (Ho–He)/He. Ho and He stand for observed and expected heterozygosities, respectively. Koehn *et al.* (1971, 1976) have discussed the properties of the *D* statistics. A negative value of D indicates excess of homozygosity and deficiency of heterozygosity while a positive value of D suggests excess of heterozygotes and deficiency of

homozygotes. The D value is expected to be zero, if the genotype frequencies are in Hardy-Weinberg equilibrium.

Although most polymorphic loci in most species of mussel populations show an excess of homozygosity (D values are negative) the pattern of the homozygosity excess is not totally random. These features could be summarized as follows:

a. *D* values are variable over enzyme loci, species and populations (Skibinski *et al.*, 1977).

b. *D* values are higher in groups of larger than in groups of smaller individuals of the same population (Koehn *et al.*, 1976; Levinton & Fundiller, 1975).

c. *D* values are higher in adults than in the juveniles of the same population (Tracey *et al.*, 1975).

2. *Macoma balthica* (L.)

The basic reproductive biology of the genus *Macoma* is not very different from *Mytilus* (Lammens, 1967). The larval and post-settlement stages are distinct with synchronous spawning, external fertilization and an age-specific mortality. Levinton (1975) studied polymorphism at two loci in eight species of *Macoma* and observed varying degrees of excess of homozygosity. One of us (R.H.G.) has followed a population of *M. balthica* from Hudson Bay intertidal region (58¼ 46′N, 94¼ 02′W) since 1970. The study area is characterized by mixed sand and boulder substrates with salinity at high tide being approximately 18‰. The average slope of the beach is 1 in 125 with mean amplitude 3.35 (see Green, 1973, for additional details). Green (1973) also observed a higher growth rate for individuals occupying a high-tide area (1.1 m above mean low water level) than the individuals in the low tide (at mean low water) area in our study site. We collected large numbers of clams from both tide levels. Using annual rings in the Walford plot method we obtained ages and a growth-rate index for individuals from both populations. These individuals were electrophoresed for a number of enzymes. Data on six polymorphic loci will be presented. Electrophoretic procedures used were based on Schaal & Anderson (1974). Table 1 shows that there is an excess of homozygosity in the two tide level populations at the six polymorphic loci (D values negative). The D values vary among loci, are consistently higher in the high-tide population than in the low-tide population. Although all electromorphs are present in both populations, allelic frequency differences between the two populations are significant for AKP, MDH and ME. Also the genotype frequency differences between populations are significant at the

TABLE 1. Locus specific statistics for polymorphic loci in two populations of Hudson Bay *Macoma balthica*.

Enzymes	Populations from tide level	No.	No. of alleles	P (H.W.)	D[1]	Population difference: Genotypic (f)	Population difference: Allelic (f)
Alkaline phosphatase (AKP)	High	87	5	>0.010	−0.229	*	*
	Low	88	5	>0.001	−0.445		
Acid phosphatase (ACP)	High	87	2	N.S.	−0.145	N.S.	N.S.
	Low	88	2	N.S.	−0.047		
Esterase (EST)	High	87	5	>0.001	−0.344	N.S.	N.S.
	Low	88	5	>0.001	−0.424		
Leucine aminopeptidase (LAP)	High	87	4	>0.001	−0.291	N.S.	N.S.
	Low	88	5	0.001	−0.414		
Malic dehydrogenase (MDH)	High	87	2	N.S.	−0.053	*	*
	Low	88	2	N.S.	−0.271		
Malic enzyme (ME)	High	87	5	>0.001	−0.507	N.S.	*
	Low	88	5	>0.001	−0.559		

*Significant, N.S.—not significant; χ^2 values of contingency.
P (H.W.) is the chance probability for H.W. equilibrium.
[1]See text for explanation.

AKP and MDH loci. This may suggest a microgeographic differentiation involving the two contiguous populations.

3. *Crassostrea virginica* (Gmelin)

Singh & Zouros (1978), Singh (1978), Zouros *et al.* (1980), Singh & Zouros (1981) and Singh (1982) studied two populations of *C. virginica*. The first is from the Malpeque Bay area of Prince Edward Island (PEI) and the second occupies the Bras d'or lakes (Cape Breton Island) of Nova Scotia. They represent two well established populations on the northernmost periphery of the distribution of this species. In this cold climate spawning is synchronized with warming temperature (20°C) in June/July and growth is limited to the summer months only. Larvae, which are produced once a year, grow to settle and form spat. Due to limited growth these oysters require up to seven years to reach market size. The distribution of five year classes of PEI populations is given in Fig. 1. There is a tremendous variation in growth rate observed at different stages of development (Singh & Zouros, 1978) including larvae (Haley *et al.*, 1975). Haley & Newkirk (1977) also demonstrated strong genetic components to growth rate. A survey of the population in May (prior to spawning) indicated that most of the 1-year-olds were too small for gonadal development and would not spawn. A high proportion of 2- and 3-year-olds were large enough to spawn. This proportion may vary depending upon growth conditions, as first spawning is dependent on size (2.5 cm long and over 5 g in weight). Almost all the over-4-year-olds were in spawning condition. It appears that the fast growing larvae take a shorter time to reach the settling size after fertilization and continue as fast-growing 1- and 2-year-olds. Conversely, the slow growing larvae settle late and continue to be slow growers (Haley & Newkirk, 1977). Growth rate in oysters, therefore, is an important feature of biological significance with strong genetic components. In an ongoing study of the oysters of Maritime Canada, we studied a number of polymorphic enzymes in two populations. Part of the results related to locus specific statistics are given in Table 2. It shows a general deficiency of heterozygotes (excess of homozygosity) in all samples. Here, the D values are locus specific and there is a decreasing trend for average D with age in the Cape Breton samples. The data also permit evaluation of the role of electrophoretic genotypes in fitness features like growth rate and mortality that are easily measured on species of molluscs. We will present these in the following sections.

Some features of the excess of homozygosity and deficiency of heterozygotes

Relatively high excess of homozygosity in smaller as compared to the larger individuals of the same population is observed in a number of reports on species of *Mytilus*. Tracey *et*

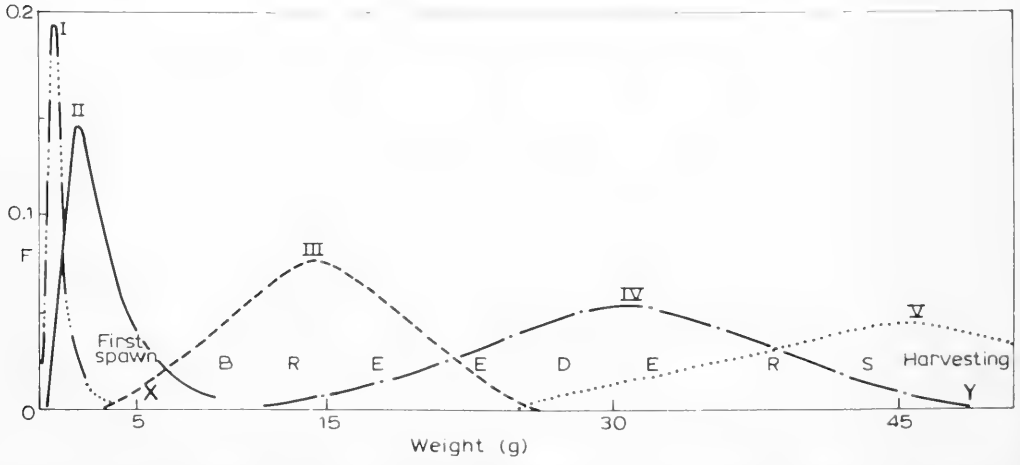

FIG. 1. Actual fitted weight distribution of various year-classes of PEI *Crassostrea virginica*. F = frequency. Note the increase in variance with increasing age and the stage to first spawning is a function of size and not age (from Singh, 1978).

TABLE 2. Locus specific statistics for five polymorphic enzyme loci in two Canadian populations of *Crassostrea virginica*. P (H.W.) is the chance probability for Hardy-Weinberg equilibrium.

	Populations Cape Breton (Singh, 1982)									P.E.I. (one-yr. olds)			
	1 yr.			2 yr.			3 yr.			1			2
Enzymes	No.	P (H.W.)	D*	No.	P (H.W.)	D*	No.	P (H.W.)	D*	No.	P (H.W.)	D*	D*
Esterase (EST)	200	0.001	−0.50	209	0.001	−0.30	203	0.001	−0.41	1616	0.001	−0.37	−0.33
Glutamate oxaloacetate transaminase (GOT)	200	N.S.	−0.07	208	N.S.	−0.10	204	0.005	+0.07	1733	N.S.	−0.01	−0.01
Phosphoglucose isomerase (PGI)	209	N.S.	0.00	209	N.S.	+0.04	206	0.001	+0.20	1764	0.019	−0.06	−0.12
Leucine aminopeptidase (LAP)	209	0.001	−0.32	209	0.001	−0.50	203	0.001	−0.36	1803	0.001	−0.44	−0.18
Phosphoglucomutase (PGM)	—	—	—	—	—	—	—	—	—	1735	0.001	−0.34	−0.39
Average	—	—	−0.23	—	—	−0.21	—	—	−0.13	—	—	−0.25	−0.20

*See text for explanation.
1. Spat collected from 1976 spawning (Zouros *et al.*, 1980).
2. Spat collected from 1974 spawning (Singh & Zouros, 1978).

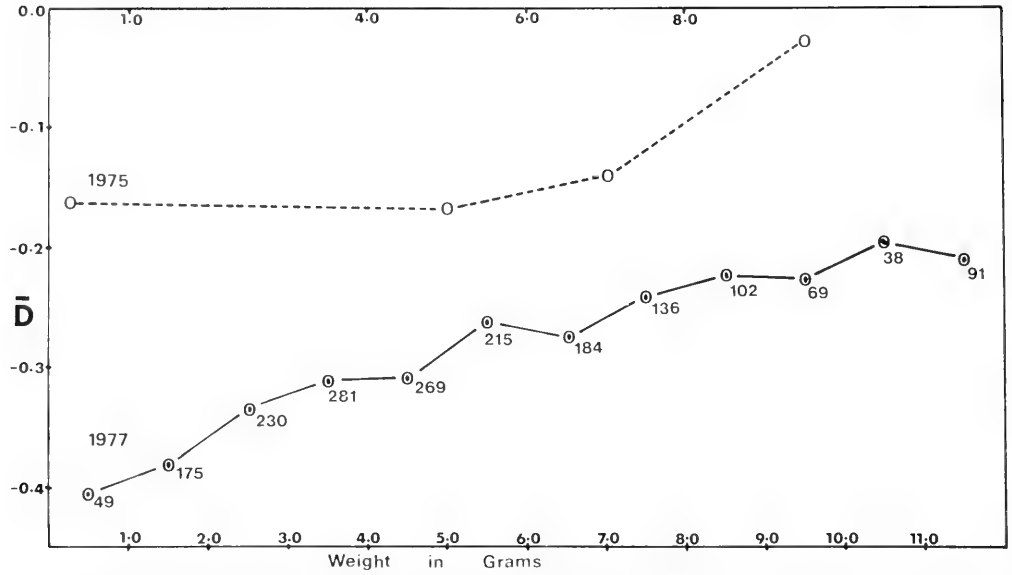

FIG. 2. Relationship between mean weight class and D (averaged over loci) in two samples of one-year-old *Crassostrea virginica* PEI population (from Singh & Zouros, 1981).

al. (1975) classified individuals into juveniles and adults and found that this excess was significantly higher in the juveniles than the adults (D for juvenile = −0.446 as compared to −0.188 for adults over two polymorphic loci). In a similar study on *Crassostrea virginica,* we (Singh & Zouros, 1978) found that there is a marked difference in weights of individuals of a given age. Also in the 1-year-olds the deficiency of heterozygotes is higher in individuals weighing less than one gram (D = −0.2116) as compared to individuals weighing over four grams (D = −0.1981). In a repeated study on the one-year-olds from the same population using large sample size and random sampling, we (Zouros *et al.*, 1980) observed that there is a strong correlation between mean weight at one year and the relative excess of homozygosity (Fig. 2). Such an observation will be expected if (1) heterozygosity is responsible for higher growth rate and/or (2) there is a selective mortality of the homozygotes during aging. We will deal with these possibilities in the following sections. Further study on three age groups (Singh, 1982) of the Cape Breton population followed our findings on PEI oysters. Analysis of four enzyme loci yielded negative values of D for one-, two- and three-year-olds. The average D (over four loci) and observed heterozygosity is higher in older than in younger age groups (Fig. 3a). These results follow Tracey *et al.* (1975) and suggest that there is preferential mortality of homozygotes during the post settlement stages. Studies on one-, two- and three-year-old oyster spat also

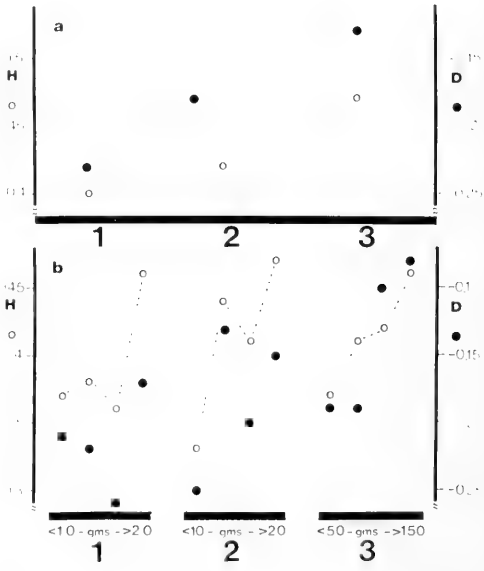

FIG. 3. Distribution of average heterozygosity (H) and D in (a) three (1, 2 and 3) year classes of Cape Breton *Crassostrea virginica* and (b) different weight categories of each year class.

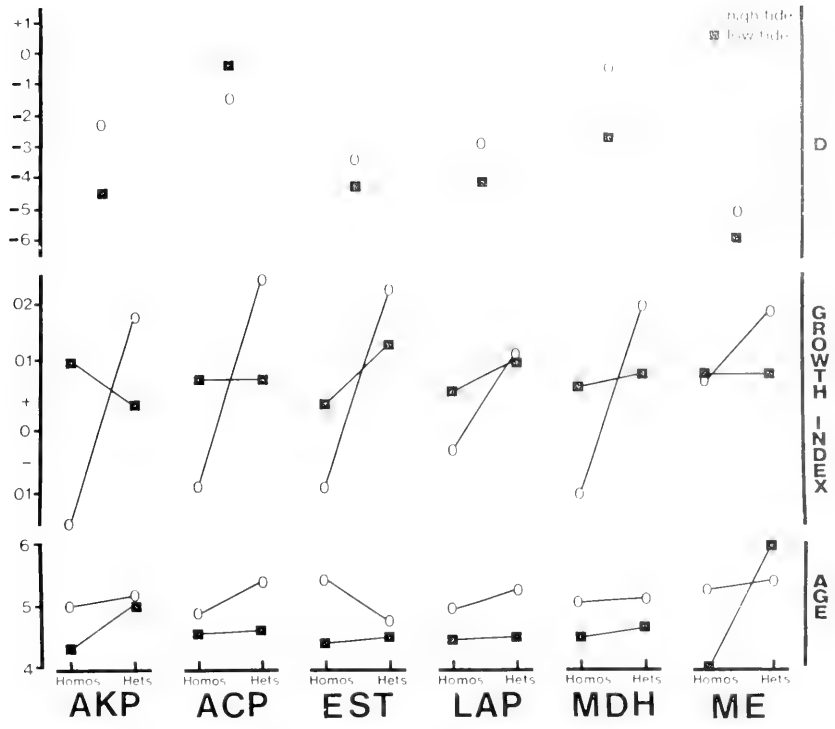

FIG. 4. Growth index and mean age (yrs.) of homozygotes and heterozygotes at six polymorphic loci in two populations of *Macoma balthica*. Estimates of D are also included for comparisons.

permitted a similar conclusion by Zouros *et al.* (1983). When the frequency of heterozygotes and the estimates of D are compared in different weight classes of the one-, two- and three-year-old oysters, it suggests an increase in heterozygosity and D with increasing mean weight in all three age groups (Fig. 3b). These observations reflect that in the American oysters and possibly in other species with excess of homozygotes, there is a genotype dependent growth rate and mortality.

Genotype dependent growth and mortality

Fig. 4 compares the growth index and age composition of homozygotes and heterozygotes of six polymorphic loci in *Macoma balthica* from two populations representing low- and high-tide areas. Green (1973) found that the high tide population has a faster growth rate than its low tide counterpart. It is evident from the figure that the mean growth index of heterozygotes is higher, at both tide levels than for homozygotes. This is true for 11 of the 12 comparisons. The probability that this pattern is by chance alone is approaching zero. Furthermore, the difference in the growth index between homozygotes and heterozygotes is much more pronounced in the fast growing high tide population than the low tide population. Also, the difference in the growth rate between homo- and heterozygotes varies from locus to locus in both populations. Such a genotype dependent growth rate is also suggested from a number of other studies (Buroker, 1979; Chaisson *et al.*, 1976; Singh & Zouros, 1978; Singh, 1982; Zouros *et al.*, 1980).

The mean ages of individuals homozygous and heterozygous at the six polymorphic loci of the two *Macoma* populations are also presented in the bottom of Fig. 4. In general the mean age of heterozygotes is higher than the mean age of the corresponding homozygotes in both populations. This trend is reflected in 11 of the 12 possible comparisons. The probability that it is by chance alone is almost zero. These results follow Zouros *et al.* (1983) and reflect on the continuous selective

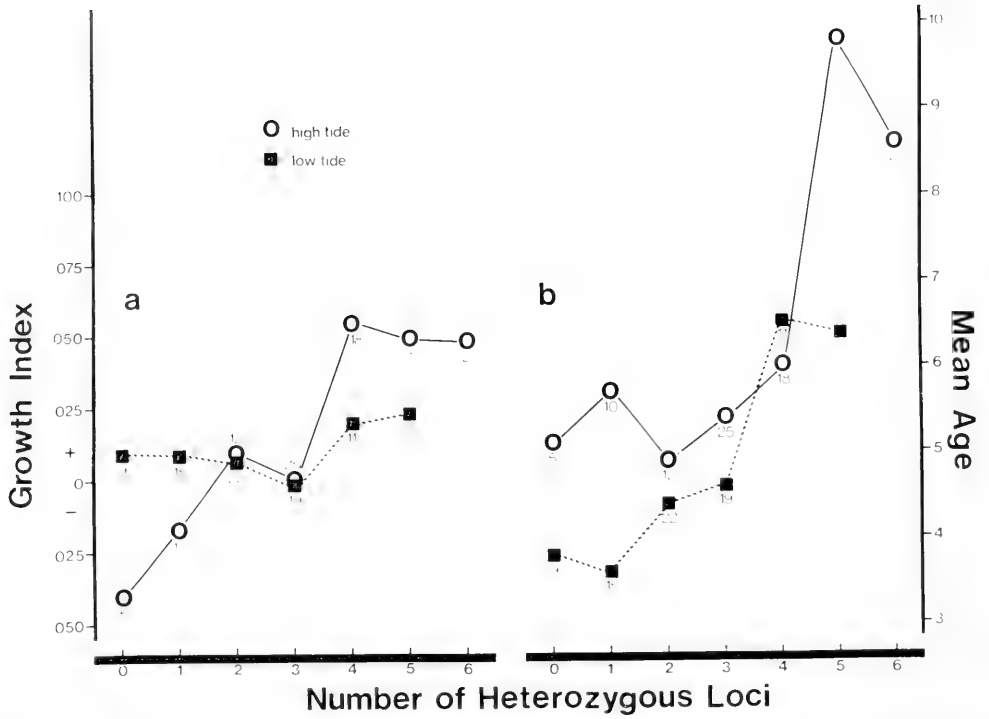

FIG. 5. Mean growth index (a) and age in yrs. (b) with varying number of heterozygous loci in high and low tide populations of *Macoma balthica*.

mortality of the homozygotes during development following settling in oysters.

The relationship between growth and mortality, and homozygosity/heterozygosity in *M. balthica* was also assessed for the six polymorphic loci by evaluating individuals with different numbers of heterozygous loci. Individuals were grouped in seven classes; heterozygotes for none of the six loci (0), any one of the six loci (1) and so on to all six loci (6). In Fig. 5 the mean growth index and age of these groups are plotted against the number of loci for which the group is heterozygous for the two populations. It suggests that the mean growth rate of an individual increases with increasing number of heterozygous loci.This relationship is more evident for the high tide (faster growing) population than for the low tide population. In a similar comparison with large sample size, Zouros *et al.* (1980) observed significant Kendall's nonparametric coefficient of rank correlation ($\tau = 0.90$) between mean weight and number of heterozygous loci in one year old PEI oysters. Furthermore, studies on one- and two-year-old oysters of the Cape Breton population (Singh, 1982) yielded significant association ($P = 0.05$) between mean transformed weight and number of heterozygous loci, for both year classes. Also the variance of individual weights reflected a decreasing trend with increasing number of heterozygous loci (Table 3). Such observations suggest that heterozygosity contributes significantly to the homogeneous fast growth of molluscs in general and oysters in particular at least during stages following settling.

The relationship between number of heterozygous loci and age in the two *M. balthica* populations is presented in Figure 5b. In general older individuals have higher heterozygosity than younger individuals, in both populations. This may be caused by selective mortality of homozygotes. A similar conclusion could also be drawn from the data on Cape Breton oysters presented in Fig. 6. It shows that the frequency of younger individuals is higher in a group representing more homozygous loci and that the frequency of older individuals is higher in the group(s) representing more heterozygous loci. Such a system of genotype dependent mortality will

TABLE 3. Weight distribution in individuals with increasing numbers of heterozygous loci in different groups of *Crassostrea virginica*.

Population	Year class	No. of heterozygous loci	Number of individuals	Mean weight (g)	Log_e of weight	
					mean	variance
PEI[1]	1	0	37	4.28	1.25	0.49
		1	169	4.78	1.37	0.43
		2	296	4.68	1.37	0.38
		3	293	5.48	1.54	0.37
		4	161	5.96	1.66	0.28
		5	40	6.44	1.77	0.35
		6	14	7.33	1.94	0.12
		7	0	—	—	—
Cape Breton[2]	1	0	29	1.11	−0.06	0.42
		1	68	1.33	0.17	0.32
		2	62	1.33	0.18	0.29
		3	35	1.33	0.18	0.25
		4	6	1.39	0.25	0.21
Cape Breton[2]	2	0	29	13.99	2.56	0.15
		1	66	12.92	2.48	0.16
		2	66	14.37	2.59	0.14
		3	38	14.40	2.60	0.14
		4	10	18.15	2.79	0.11

[1]Based on Zouros *et al.* (1980).
[2]Based on Singh (1982).

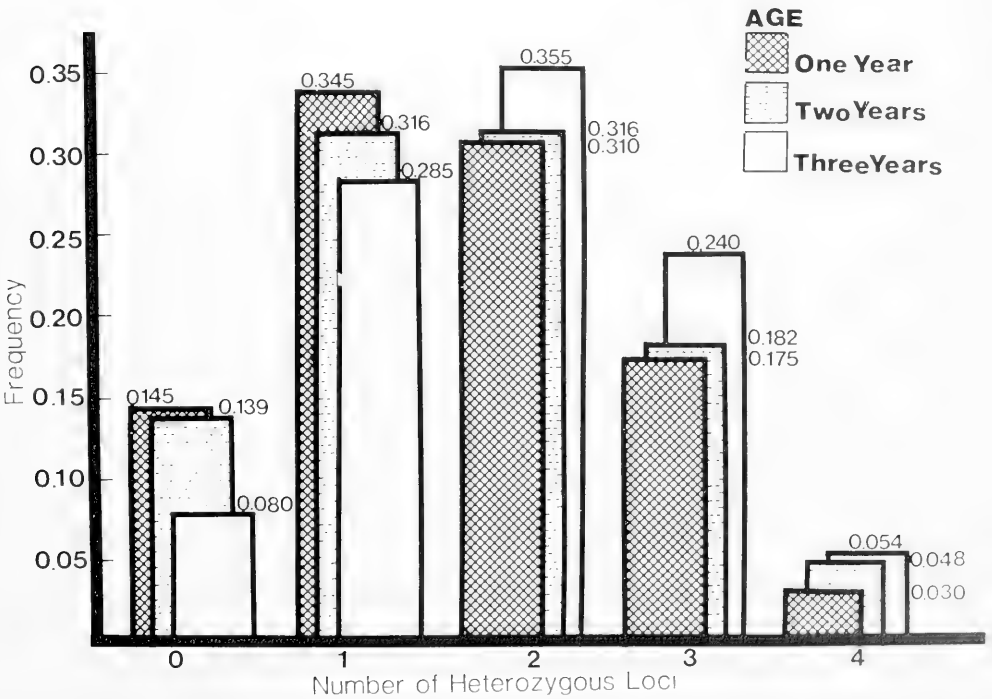

FIG. 6. Frequency of different age categories in groups representing different numbers of heterozygous loci in the Cape Breton *Crassostrea virginica*.

yield a cline for the most common allele at a given locus. These alleles are expected to be present more as homozygotes in the population, thus with higher mortality of homozygotes, the frequency of the common alleles will decline through age. In two of the four loci studied in three year classes of oysters, the expected cline for the allelic frequencies was found to be significant. Although it requires additional study for confirmation, results on the declining frequency of common alleles through age at GOT and EST loci support the hypothesis of selective mortalty of homozygotes through aging.

Origin of excess homozygosity

Haley & Newkirk (1977) have reported that there is a strong genetic component to oyster larval growth. An early settled spat (presumably a result of faster growing larvae) continues as a faster growing oyster than the late settled spat (from slower growing larvae). Results presented in the preceding pages suggest that heterozygotes have higher fitness values (faster growth rate and lower mortality) from spat stage to adults. They grow up to reach spawning size sooner and release relatively more gametes, primarily because of their larger size at a given age than their homozygous counterparts. The relative fitness advantage of heterozygotes is however, evident only in stages following settling. The origin of the excess of homozygosity along with associations between heterozygosity and post settlement growth rate could be explained by a form of balancing selection. It involves fitness reversal of heterozygotes during the pelagic larval phase. This may be caused by higher mortality of fast growing heterozygote larvae due to their higher food (phytoplankton) requirement, as compared to the slow growing homozygotes. This preferential mortality of heterozygotes will lead to the excess of homozygosity in the settled spat and resulting oyster population.

A number of indirect observations support this view. First, the relative mortality of heterozygotes (fast growers) during the larval phase is expected to vary from year to year depending on climatic conditions, particularly the relative abundance of phytoplankton. In a year with a poor phytoplankton bloom fast growing heterozygotes with a higher food requirement will face relatively higher mortality. This may account for the poor spat years experienced by spat collectors and oyster growers, where relatively few larvae grow to form spat.Spat from such years are also slow growers and take a longer time to reach market size (Galtsoff, 1964). Second, when larvae from two crosses (fast × fast and slow × slow growers from a given age group) are grown in a single tank with no/little food supplement, only the larvae from slow × slow cross survive to settle and form spat. These observations are consistent with preferential mortality of fast growing heterozygotes during larval phases because of their higher food requirements. The reproductive potential and biology of molluscs in general and oysters in particular is not incompatible with this model. Each individual may release millions of gametes that may form larvae and only a small fraction of these settle to form spat. They can accommodate a very high level of larval and adult mortality in every generation.

The phenomenon of the fitness reversal based on density or during different phases of the life cycle has been demonstrated in a number of organisms including plants (Clegg *et al.*, 1978). Considering the life cycle similarities between molluscs and plants (Williams, 1975), we favour the fitness reversal of heterozygotes in two very distinct and different phases of the life cycle of molluscs to be responsible for the origin of the excess of homozygosity and maintenance of genetic variability in the natural populations of these species. A basic diagram of their life cycle and generalized model for the phenomenon is given in Fig. 7. Zouros & Foltz (1984) have also considered a number of other models in some detail and their implications.

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REFERENCES CITED

AYALA, F. J., HEDGECOCK, D., ZUMWALT, G. S. & VALENTINE, J. W., 1973, Genetic variation in *Tridacna maxima*, an ecological analog of some

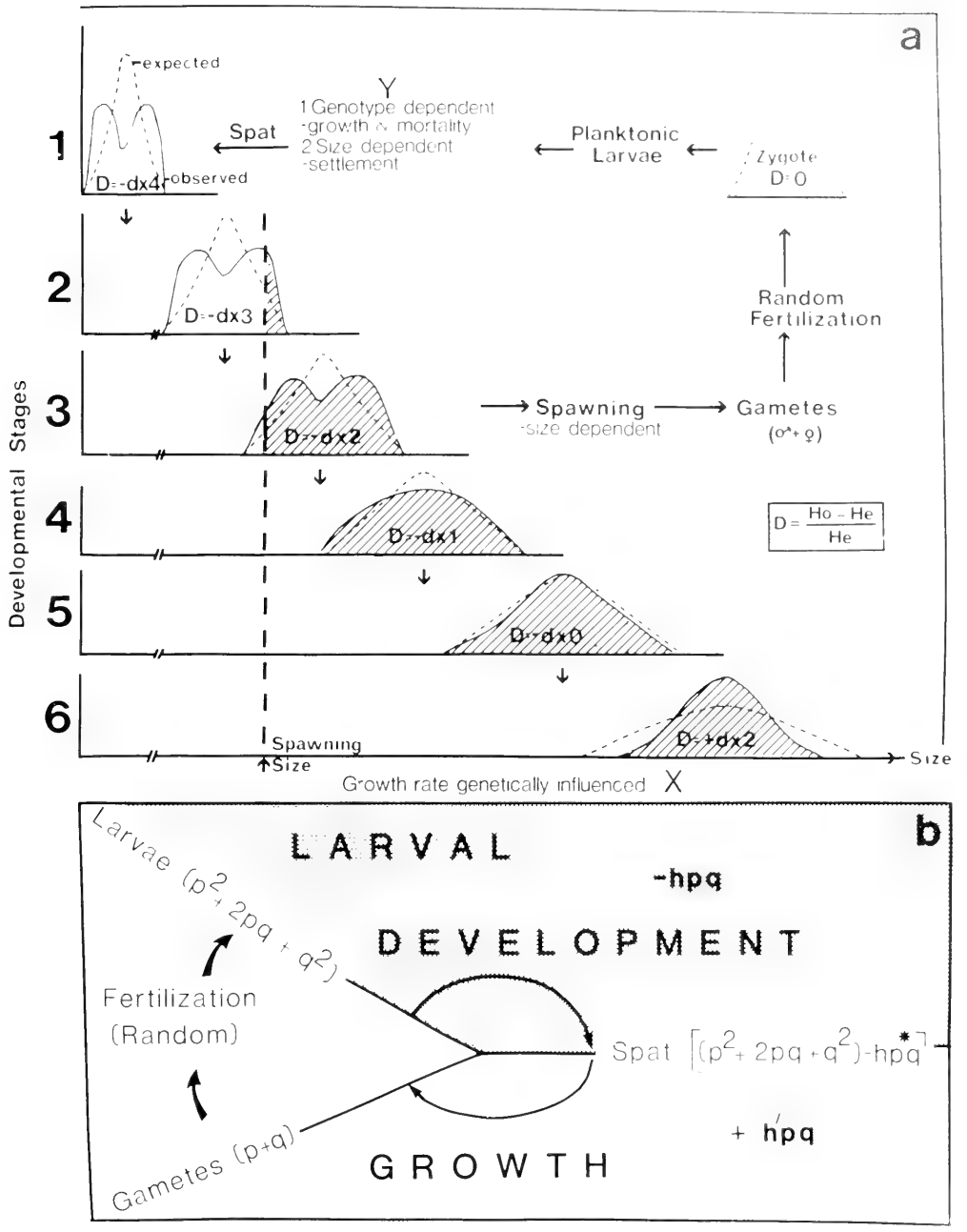

FIG. 7. A generalized model of the mollusc life cycle showing origin of the excess of homozygosity and maintenance of genetic variation. a. shows genetically determined growth and mortality of planktonic larvae (Y) and sedentary individuals (X) at different developmental stages. Note that the difference in observed and expected curves at stage 1 is caused by preferential mortality of heterozygotes ($-d \times 4$). Changes in subsequent generations result from continuous mortality of homozygotes. It is responsible for increasing trend of D with age. b. a simplified model, with fitness reversal of heterozygotes between larval ($-hpq$) and sedentary ($+h'pq$) phases of the life cycle, responsible for origin of the excess of homozygosity and maintenance of genetic variation in molluscs with pelagic larvae.

unsuccessful evolutionary lineages. *Evolution,* 27: 177–191.

BAYNE, B. L., 1965, Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). *Ophelia,* 2: 1–47.

BOYER, J. F., 1974, Clinal and size dependent variation at the leucine aminopeptidase locus in *Mytilus edulis. Biological Bulletin,* 147: 535–549.

BUROKER, N. E., 1979, Overdominance of a muscle protein (Mp-1) locus in the Japanese oyster, *Crassostrea gigas* (Ostreidae). *Journal of the Fisheries Research Board of Canada,* 36: 1313–1318.

BUROKER, N. E., HERSCHBERGER, W. K. & CHEW, K. K., 1975, Genetic variation in the Pacific oyster, *Crassostrea gigas. Journal of the Fisheries Research Board of Canada,* 32: 2471–2477.

CHAISSON, R. E., SERUNIAN, L. A. & SCHOPF, T. J. M., 1976, Allozyme variation between two marshes and possible heterozygote superiority within a marsh in the bivalve *Modiolus demissus. Biological Bulletin,* 151: 404. Abstract.

CLEGG, M. T., KAHLER, A. L. & ALLARD, R. W., 1978, Estimation of life cycle components of selection in an experimental plant population. *Genetics,* 89: 765–792.

CRISP, D. J., 1977, Genetic consequences of different reproductive strategies in marine invertebrates. In: BATTAGLIA, B. & BEARDMORE, J. A., ed., *Marine organisms: genetics, ecology and evolution.* Plenum, New York, p. 257–273.

FIELD, I. A., 1922, Biology and economic value of the sea mussel *Mytilus edulis. Bulletin of the United States Bureau of Fisheries,* 38: 127–260.

FRIP, Y. J. & CATEN, C. E., 1971, Genotype-environment interactions in *Schizophyllum commune. Heredity,* 27: 393–407.

GALTSOFF, P. S., 1964, The American oyster *Crassostrea virginica* Gmelin. *United States Department of the Interior Fishery Bulletin of the Fish and Wildlife Service,* 64: iii + 480 p.

GARTNER-KEPKAY, K. E., DICKIE, L. M., FREEMAN, K. G. & ZOUROS, E., 1980, Genetic differences and environments of mussel populations in the Maritime provinces. *Canadian Journal of Fisheries and Aquatic Sciences,* 37: 775–782.

GREEN, R. H., 1973, Growth and mortality in an arctic intertidal population of *Macoma balthica* (Pelecypoda, Tellinidae). *Journal of the Fisheries Research Board of Canada,* 30: 1345–1348.

HALEY, L. E., NEWKIRK, G. F., WAUGH, D. N. & DOYLE, R. W., 1975, A report on the quantitative genetics of growth and survivorship of the American oyster, *Crassostrea virginica* under laboratory conditions. *10th European Symposium on Marine Biology,* Ostend, 1: 221–228.

HALEY, L. E. & NEWKIRK, G. F., 1977, Selecting oysters for faster growth. *Proceedings of the Eighth Annual Meeting, World Mariculture Society,* p. 555–565.

HEDRICK, P. W., GINEVAN, M. E. & EWING, E. P., 1976, Genetic polymorphism in heterogeneous environments. *Annual Review of Ecology and Systematics,* 7: 1–32.

HORNBACH, D. J., McLEOD, N. J., GUTTMAN, M. I. & SEILKOP, S. K., 1980, Genetic and morphological variation in the freshwater clam, *Sphaerium* (Bivalvia Sphaeriidae). *Journal of Molluscan Studies,* 46: 158–170.

JOHNSON, A. G. & UTTER, F. M., 1973, Electrophoretic variants of aspartate aminotransferase of the bay mussel, *Mytilus edulis* (Linnaeus, 1758). *Comparative Biochemistry and Physiology,* 44B: 317–323.

KARTAVTSEV, Yu. F. & PUDOVKIN, A. I., 1977, Genetic variability of the mussel *Crenomytilus grayanus* (Dunker) in *Abstracts of Proceedings of the Organizational Conference of the Far East Section of the N.I. Vavilov All-Union State Institute of Selection,* p. 9–11. In Russian.

KOEHN, R. K., MILKMAN, R. & MITTON, J. B., 1976, Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel *Mytilus edulis. Evolution,* 30: 2–32.

KOEHN, R. K. & MITTON, J. B., 1972, Population genetics of marine pelecypods. I. Ecological heterogeneity and adaptive strategy at an enzyme locus. *American Naturalist,* 106: 47–56.

KOEHN, R. K., PEREZ, J. E. & MERRITT, R. B., 1971, Esterase enzyme function and genetical structure of populations of the freshwater fish, *Notropis stramineus. American Naturalist,* 105: 51–69.

KOEHN, R. K., TURANO, F. J. & MITTON, J. B., 1973, Population genetics of marine pelecypods. II. Genetic differences in microhabitats of *Modiolus demissus. Evolution,* 27: 100–105.

LAMMENS, J. J., 1967, Growth and reproduction in a tidal flat population of *Macoma balthica. Netherlands Journal of Sea Research,* 3: 315–382.

LASSEN, H. H. & TURANO, F. J., 1978, Clinal variation of heterozygote deficit at the lap-locus in *Mytilus edulis. Marine Biology,* 49: 245–254.

LAVEE, D. & RITTE, V., 1977, Genetic parameters as indices of preadaptation for successful colonization in mytilids (Mollusca Pelecypoda). *Israel Journal of Zoology,* 26: 259. Abstract.

LEVINTON, J. S., 1975, Levels of genetic polymorphism at two enzyme encoding loci in eight species of the genus *Macoma* (Mollusca: Bivalvia). *Marine Biology,* 33: 41–47.

LEVINTON, J. S. & FUNDILLER, D. L., 1975, An ecological and physiological approach to the study of biochemical polymorphisms. In BARNES, H., ed., *Proceedings of the Ninth European Marine Biology Symposium.* Aberdeen University Press, Scotland, p. 165–178.

MILKMAN, R. & BEATY, L. D., 1970, Large-scale electrophoretic studies of allelic variation in *Mytilus edulis. Biological Bulletin,* 139: 4–30. Abstract.

MITTON, J. B., KOEHN, R. K. & PROUT, T., 1973, Population genetics of marine pelecypods. III.

Epistasis between functionally related isoenzmes of *Mytilus edulis*. *Genetics*, 73: 487–496.

NEVO, E., 1978, Genetic variation in natural populations. Patterns and theory. *Theoretical Population Biology*, 13: 121–177.

SCHAAL, B. A. & ANDERSON, W. W., 1974, An outline of techniques for starch gel electrophoresis of enzymes from the American Oyster *Crassostrea virginica* Gmelin. *Georgia Marine Science Center Technical Report Series* No. 74-3.

SINGH, S. M., 1978, A model for the maintenance of genetic variability and its utilization in genetic improvement of oyster populations. *Aquaculture*, 15: 289–295.

SINGH, S. M., 1982, Enzyme heterozygosity associated with growth at different developmental stages in oysters. *Canadian Journal of Genetics and Cytology*, 24: 451–458.

SINGH, S. M. & ZOUROS, E., 1978, Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). *Evolution*, 32: 342–353.

SINGH, S. M. & ZOUROS, E., 1981, Genetics of growth rate in oysters and its implications for aquaculture. *Canadian Journal of Genetics and Cytology*, 23: 119–130.

SKIBINSKI, D. O. F., AHMAD, M. & BEARDMORE, J. A., 1978, Genetic evidence for naturally occurring hybrids between *Mytilus edulis* and *Mytilus galloprovincialis*. *Evolution*, 32: 354–364.

SKIBINSKI, D. O. F. & BEARDMORE, J. A., 1979, A genetic study of intergradation between *Mytilus edulis* and *Mytilus galloprovincialis*. *Experientia*, 35: 1442–1444.

SKIBINSKI, D. O. F., BEARDMORE, J. A. & AHMAD, M., 1977. Genetic aids to the study of closely related taxa of the genus *Mytilus*. In BATTAGLIA, B. & BEARDMORE, J. A. ed., *Marine organisms: genetics, ecology and evolution*. Plenum, New York, p. 469–486.

STEBBINS, G. L., 1970, Variation and evolution in plants: progress during the past twenty years. In HECHT, M. K. & STEERE, W. C. ed., *Essays in evolution and genetics in honour of Theodosius Dobzhanski*. Appleton-Century-Crofts, p. 173–208.

TRACEY, M. L., BELLET, N. F. & GRAVEM, C. D., 1975, Excess allozyme homozygosity and breeding population structure in the mussel *Mytilus californianus*. *Marine Biology*, 32: 303–311.

WILLIAMS, G. C., 1975, *Sex and evolution*. Princeton University Press, p. 35–43.

ZOUROS, E. & FOLTZ, D. W., 1984, Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacologia*, 25: 583–591.

ZOUROS, E., SINGH, S. M., FOLTZ, D. W. & MALLET, A., 1983, Post-settlement viability in the American oyster (*Crassostrea virginica*): an overdominant phenotype. *Genetical Research, Cambridge*, 41: 259–270.

ZOUROS, E., SINGH, S. M. & MILES, H. E., 1980, Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution*, 34: 856–867.

MALACOLOGIA, 1984, 25(2): 583–591

POSSIBLE EXPLANATIONS OF HETEROZYGOTE DEFICIENCY IN BIVALVE MOLLUSCS

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ABSTRACT

There have been many reports of heterozygote deficiency at electrophoretically-detected loci in natural populations of bivalve molluscs. This phenomenon is prevalent in an American oyster (*Crassostrea virginica*) population that we have studied for the last five years (Singh & Zouros, 1978; Zouros *et al.*, 1980). Elsewhere (Zouros *et al.*, 1980), we have presented arguments against the hypotheses of inbreeding and population mixture as an explanation of the observed deficiencies of heterozygotes in *C. virginica*. Here, we examine models of selection and non-random mating that may generate heterozygote deficiency without genetic differentiation. In particular, we consider the following three models: (1) Viability rates are reversed from the planktonic stage to the post-settlement stage. Under this model, heterozygote deficiency may appear when the population is scored after settlement but before adult selection is completed. This situation may occur if the allele selected against in the larval stage is dominant (in its selective effect) over the allele selected for. Therefore, underdominance in viability is not a necessary condition for heterozygote deficiency. (2) Viability selection is confined to the larval stage and is compensated by differential fecundity in the adult stage. This model may generate post-settlement heterozygote deficiency and, again, for this event to occur there is no need for underdominance in larval viabilities. (3) Spawning time is genotype-dependent. When homozygotes spawn at different times than heterozygotes, there will occur in the population a heterozygote deficiency whose equilibrium value depends on the allele frequency and the coefficient of overlap between the spawning times of heterozygotes and homozygotes. In general, the magnitude of the heterozygote deficiency generated by this model is inversely proportional to the amount of overlap in spawning between genotypes.

The models are based on the observation that, in *C. virginica,* heterozygotes attain larger size (Zouros *et al.,* 1980), thus producing more gametes than homozygotes, and may also have lower post-settlement mortality rates (Zouros, *et al.,* 1983). The plausibility of the models is judged by comparing the predicted heterozygote deficiencies to the values commonly reported in the literature for *C. virginica* and other species of bivalves.

Key words: electrophoresis; molluscs; heterozygosity; selection; non-random mating.

INTRODUCTION

Numerous studies of natural populations of bivalve molluscs have shown heterozygote deficiency at enzyme loci. (The term "heterozygote deficiency" refers to a polymorphic locus for which the observed number of heterozygous individuals is significantly lower than the Hardy-Weinberg expectations.) Heterozygote deficiencies at one or more loci have been reported for over two dozen bivalve species: *Brachidontes rostratus* (Colgan, 1981), *B. variabilis* (Lavee & Ritte, 1977), *Mytilus californianus* (Tracey *et al.,* 1975; Levinton & Fundiller, 1975), *M. edulis* (Milkman & Beaty, 1970; Koehn & Mitton, 1972; Mitton *et al.,* 1973; Johnson & Utter, 1973; Boyer, 1974; Koehn *et al.,* 1976; Lassen & Turano, 1978; Gartner-Kepkay *et al.,* 1980), *Crenomytilus grayanus* (Kartavtsev, 1978), *Modiolus auriculatus* (Lavee & Ritte, 1977), *M. demissus* (Koehn & Mitton, 1972; Koehn *et al.,* 1973), *Ostrea edulis* (Wilkins & Mathers, 1973), *Crassostrea angulata* (Buroker *et al.,* 1979a), *C. belcheri* (Buroker *et al.,* 1979b), *C. gigas* (Buroker *et al.,* 1975, 1979a; Fujio, 1979; Gosling, 1982), *C. iredalei, C. rhizophorae, C. rivularis* (Buroker *et al.,* 1979b), *C. virginica* (Singh & Zouros, 1978; Zouros *et al.,* 1980), *Saccostrea commercialis, S. glomerata* (Buroker *et al.,* 1979a), *S. malabonensis* (Buroker *et al.,*

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1979b), *Tridacna maxima* (Ayala *et al.,* 1973), *Cerastoderma edule* (Beaumont *et al.,* 1980), *Chlamys opercularis* (Beaumont, 1982), *Tapes decussatus* (Wilkins & Mathers, 1974), *Scrobicularia plana* (Skibinski, McNee & Beardmore, 1978), *Ensis ensis* (Wilkins & Mathers, 1974), *Sphaerium occidentale, S. simile,* and *S. striatinum* (Hornbach *et al.,* 1980).

Several explanations have been offered to account for these results, including self-fertilization (Hornbach *et al.,* 1980), null alleles (Milkman & Beaty, 1970), scoring bias (Ayala *et al.,* 1973; Buroker *et al.,* 1975), the Wahlund effect or population subdivision (Tracey *et al.,* 1975; Koehn *et al.,* 1976; Kartavtsev, 1978), and selection (Koehn & Mitton, 1972; Koehn *et al.,* 1973; Boyer, 1974; Beaumont *et al.,* 1980; Gartner-Kepkay *et al.,* 1980; Colgan, 1981). One pattern that has emerged from multi-locus studies of bivalve populations is that within a single species, different loci do not show the same deficiency of heterozygotes. This result would appear to rule out self-fertilization or other forms of close inbreeding as an explanation of heterozygote deficiency in bivalve molluscs, even for species with simultaneous hermaphroditism, such as sphaeriid clams. Of course, this explanation could be salvaged by postulating a role for selection in determining genotype frequencies, in addition to inbreeding. Similarly, null alleles appear to be an unlikely explanation for heterozygote deficiency, because several large surveys have failed to discover many presumed null homozygotes (Koehn *et al.,* 1976; Zouros *et al.,* 1980). Again, however, this result could be explained by selection, if the null allele was advantageous in the heterozygote but lethal or nearly lethal in the homozygote. This problem was considered by Zouros *et al.* (1980), who concluded that the null-allele hypothesis would require either large mutation rates or biologically unrealistic levels of heterozygote advantage. The Wahlund effect may explain some cases of heterozygote deficiency, particularly for species in which allele frequencies change drastically over small geographic distances (e.g., *Mytilus edulis*: Koehn *et al.,* 1976), but it cannot account for the marked heterozygote deficiencies observed in other bivalve species such as *C. virginica,* which are genetically homogeneous over large distances (Groue & Lester, 1982). It is also possible that some "species" of bivalves are actually complexes of sibling or cryptic species between which some hybridization occurs. For example, *Mytilus edulis* and *M. galloprovincialis* coexist and interbreed in certain areas of the British Isles (Skibinski *et al.,* 1977; Skibinski, Ahmad & Beardmore, 1978; Skibinski & Beardmore, 1979; Gosling & Wilkins, 1981). In addition to generating large deficiencies of heterozygotes, hybridization between cryptic species should cause marked gametic disequilibrium between loci. However, there is no evidence for the occurrence of generalized gametic disequilibria, at least in the American oyster (Foltz *et al.,* 1983).

Thus, by a process of elimination we are left with the possibility of natural selection. There is, moreover, evidence that selection may be more than just an explanation of last resort. Several studies of marine bivalves have indicated that heterozygote deficiencies are more pronounced in small size classes (Koehn *et al.,* 1973; Tracey *et al.,* 1975; Chaisson *et al.,* 1976; Koehn *et al.,* 1976). This result may suggest that heterozygotes have higher viabilities than homozygotes, at least after settlement, although differential recruitment (Tracey *et al.,* 1975; Milkman & Koehn, 1977) or genotype-dependent growth rates (Singh & Zouros, 1978; Zouros *et al.,* 1980) could also account for the data. Elsewhere (Zouros *et al.,* 1983), we have studied genotype frequencies in age cohorts of *Crassostrea virginica* (Gmelin, 1791) and concluded that the data are consistent with the hypothesis that heterozygotes at several enzyme loci have higher post-settlement viability rates, compared to the homozygotes.

In this paper, we examine several theoretical models of selection and non-random mating. Our focus is on the degree of selective differences or the degree of non-random mating necessary to produce heterozygote deficiencies of the magnitude commonly observed among bivalve populations, subject to the constraint that the polymorphism be maintained (i.e. the common allele must occur at a frequency less than 0.95). Our main finding is that the maintenance of heterozygote deficiency by natural selection requires rather stringent conditions. The plausibility of the genotype-dependent spawning models as an explanation of the observed levels of heterozygote deficiency in bivalve populations remains to be evaluated.

PRE-SETTLEMENT VIABILITY SELECTION

A summary of the notation used in the selection models is provided in Table 1.

TABLE 1. Notation used in models of viability and fecundity selection.

Symbol	Explanation
A_1, A_2	Alternate alleles at a locus
p, q	Frequencies of alleles A_1 and A_2, respectively
p', q'	Allele frequencies after selection
$\hat{p}$, $\hat{q}$	Equilibrium allele frequencies after selection
p_s, q_s	Allele frequencies among settling larvae
D_s	Deficiency of heterozygotes among settling larvae
s	Viability selection coefficient of A_2A_2 individuals in pre-settlement stage
h	Degree of dominance (in selective effect) of A_2 allele in pre-settlement stage
$\bar{w}$	Mean fitness of population after selection
t	Viability selection coefficient of A_2A_2 individuals in post-settlement stage
k, K	Degree of dominance (in selective effect) of A_2 allele in post-settlement stage
T	Fecundity selection coefficient of A_2A_2 individuals in post-settlement stage
$\bar{w}^*$	Mean fitness of settling larvae

Assume that larvae are produced by random mating and that p and q are the frequencies in the parental population of the two alleles (A_1 and A_2, respectively) segregating at locus A ($p + q = 1$). Also assume that the larval viabilities are as shown below:

Genotype	A_1A_1	A_1A_2	A_2A_2
Viability	1	$1 - hs$	$1 - s$

subject to the constraints that $0 < s < 1$ and that $hs < 1$. The allele frequencies at the time of settlement will be

$$p' = \frac{p^2 + pq(1 - hs)}{\bar{w}}$$

$$\text{and } q' = \frac{q^2(1 - s) + pq(1 - hs)}{\bar{w}} \qquad (1)$$

where $\bar{w} = 1 - sq(2hp + q)$. The frequency of heterozygotes will be $2pq(1 - hs)/\bar{w}$. For heterozygote deficiency, we require that

$$\frac{2pq(1 - hs)}{\bar{w}} < \frac{2\{p^2 + pq(1 - hs)\}\{q^2(1 - s) + pq(1 - hs)\}}{\bar{w}^2} \qquad (2)$$

which reduces to $h(2 - hs) > 1$. This inequality is plotted in Fig. 1. It may be noted that underdominance is not the only condition that may cause heterozygote deficiency. Dominance of the allele with the lower homozygote viability will produce the same result even though, for fixed s, larger deficiencies in the number of heterozygotes will be expected in the case of underdominance than in the case of simple dominance of the deleterious allele. Also note that, when unopposed, this selective regime will lead to the eventual extinction of the A_2 allele. We are, therefore, forced to consider more complex models of selection.

DIFFERENT PRE-SETTLEMENT AND POST-SETTLEMENT VIABILITIES

In this section, we maintain the pre-settlement viability parameters as given in the previous section, but introduce new viabilities for the post-settlement stage:

Genotype	A_1A_1	A_1A_2	A_2A_2
Post-settlement viability	1	$1 - kt$	$1 - t$

subject to the constraints that $t < 1$ and $kt < 1$. The objective is to relate the viability parameters in such a way that the polymorphism will be maintained and there will be a deficiency of heterozygotes in the population. The frequency of allele A_2, q', after one generation of selection will be

$$q' = \frac{q^2(1 - s)(1 - t) + pq(1 - hs)(1 - kt)}{p^2 + 2pq(1 - hs)(1 - kt) + q^2(1 - s)(1 - t)}. \qquad (3)$$

At equilibrium, $q = q' = \hat{q}$, which gives

$$\hat{q} = \frac{a}{a - b} \quad \text{and } \hat{p} = \frac{b}{a - b}, \qquad (4)$$

where $a = hskt - hs - kt$ and $b = st - s - t$. The polymorphism will be maintained if there is an overall overdominance, that is, if

$$(1 - hs)(1 - kt) > 1 \quad \text{and}$$

$$(1 - hs)(1 - kt) > (1 - s)(1 - t) \qquad (5)$$

which reduce to $a > 0$ and $a > b$. For $0 < q < 1$, we also require that $b < 0$. The conditions for polymorphism are, therefore, $a > 0$ and $b < 0$.

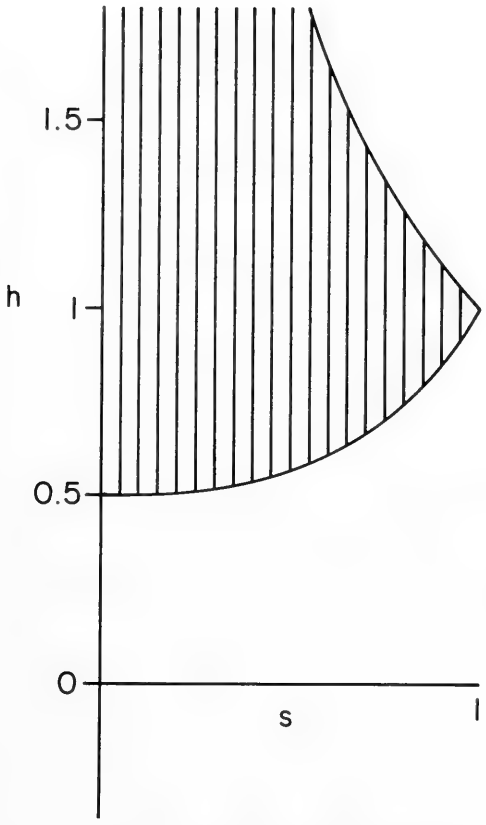

FIG. 1. Values of h and s which satisfy the inequality $h(2 - hs) > 1$ (and also the inequalities $s < 1$ and $hs < 1$) are shown in the hatched region. Overdominance in viability obtains when $h < 0$; for $0 \leq h < 0.5$ there is dominance of the allele with the higher homozygote viability; for $0.5 \leq h < 1$ there is dominance of the allele with the lower homozygote viability; for $h \geq 1$ there is underdominance in viability.

For heterozygote deficiency, we require that

$$\frac{2\hat{p}\hat{q}(1 - hs)(1 - kt)}{\bar{w}} < 2\hat{p}\hat{q} \qquad (6)$$

where $\bar{w}$ is the denominator of the right part of expression (3) at equilibrium, which can be written as

$$\bar{w} = 1 + 2a\hat{p}\hat{q} + b\hat{q}^2 \qquad (7)$$

and inequality (6) reduces to

$$a < 2a\hat{p}\hat{q} + b\hat{q}^2 \qquad (8)$$

Inserting $\hat{q} = a/(a - b)$ and $\hat{p} = -b/(a - b)$ in expression (8) leads to $ab > a^2 + b^2$, which is unattainable. We conclude that this model cannot produce a deficiency in the number of heterozygotes at the completion of the life cycle. Deficiency in the number of heterozygotes may, however, appear if the population is scored at the completion of the larval stage (i.e. at settlement). If we define $D_s = (H_o - H_e)/H_e$, where H_o is the observed number of heterozygotes at settlement and H_e is the number of heterozygotes expected if the population were in Hardy-Weinberg equilibrium, then it can be shown that the heterozygote deficiency at settlement D_s is given by

$$D_s = \frac{\{1 - h(2 - hs)\}\, s\hat{p}\hat{q}}{(1 - hs\hat{q})(1 - s\hat{q} - hs\hat{p})}. \qquad (9)$$

As expected, the sign of D_s depends solely on the viability parameters of the larval stage and is negative when $h(2 - hs) > 1$, as found in the first section. However, the magnitude of D_s is a function of both larval and adult viabilities.

In Fig. 2, we have plotted the magnitude of D_s as a function of hs and kt. Only values of hs and kt below the solid line will maintain the polymorphism, that is, produce allele frequencies at settlement (defined as p_s and q_s) which both fall in the range (0.05, 0.95). These frequencies are related to the adult (equilibrium) frequencies by the following expressions

$$p_s = \frac{\hat{p}(1 - hs\hat{q})}{\bar{w}^*} \text{ and } q_s = \frac{\hat{q}(1 - s\hat{q} - hs\hat{p})}{\bar{w}^*} \qquad (10)$$

where $\bar{w}^* = 1 - s\hat{q}(2h\hat{p} + \hat{q})$. This figure was generated by evaluating expression (9) for several thousand combinations of h, s, k, and t, subject to the constraints given above. The dashed lines indicate regions of the parameter space which yield various values of D_s. The magnitude of D_s depends largely on the pre-settlement viability of the heterozygote, $1 - hs$. This result is expected, because D_s itself is measured at settlement, before post-settlement viability differences have been expressed. Large deficiencies of heterozygotes (D_s less than -0.4) result only when heterozygotes have low viability in the pre-settlement stage ($1 - hs < .44$) but high viability in the post-settlement stage ($1 - kt > 2.2$). These viabilities are relative to the viability of the A_1A_1 homozygote. Note also that for the maintenance of polymorphism we require either that both hs and kt be negative (in which case D_s would most likely be positive) or that they have opposite signs; i.e. that

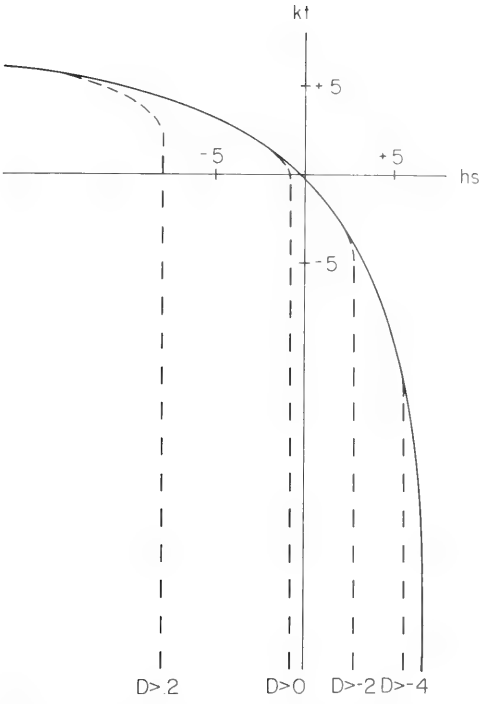

FIG. 2. Values of *hs* and *kt* which maintain a two-allele polymorphism (below solid line) and produce a heterozygote deficiency (measured by *D*). The magnitude of *D* is indicated by the dashed lines. See text for explanation of the model.

there should be a negative correlation between pre-settlement and post-settlement viabilities.

DIFFERENTIAL LARVAL VIABILITIES AND DIFFERENTIAL ADULT FECUNDITIES

The important difference between this model and the one in the previous section is that deficiencies of heterozygotes generated by differential viabilities in the pre-settlement stage will continue to exist among the adults, because genotype frequencies among newly-settled larvae and among adults remain the same. This model also appears to be more realistic, considering the facts that mortalities are very high during the planktonic (larval) stage, but less so during the adult stage, and that fecundities may vary dramatically among adults of different size and age. Let *s* and *h* be the viability parameters as defined in the previous sections, and let the relative fecundities be as follows:

Genotype	A_1A_1	A_1A_2	A_2A_2
Relative fecundity	1	$1 - KT$	$1 - T$

where upper-case letters are used to distinguish the relative fecundities from the relative viabilities employed in the previous section. Again, we require that $T < 1$ and $KT < 1$. Under this model, the equilibrium allele frequencies are

$$f(A_2) = \hat{q} = A/(2A - B) \quad \text{and}$$

$$f(A_1) = \hat{p} = (A - B)/(2A - B), \qquad (11)$$

where $A = hsKT - hs - KT$ and $B = sT - s - T$. The conditions for an overall overdominance (and, therefore, maintenance of the polymorphism) are $A > 0$ and $A > B$. The deficiency of heterozygotes at the end of the larval period is given by expression (9), as before. In Fig. 3, we have plotted the magnitude of D_s as a function of *hs* and *KT*. As in the earlier model, the requirement for obtaining heterozygote deficiencies of a magnitude comparable to those commonly reported in the literature for the American oyster and other bivalve species ($D_s < -0.4$) is low viability in the pre-settlement stage ($hs > .56$) coupled with high fecundity in the adult stage ($KT < -2.4$). Thus, the conditions required to generate large negative D_s values under this model are just as stringent as those required under the previous model. Also, negative D_s values of relatively large magnitude would require positive *hs* and negative *KT*; i.e., a negative correlation between heterozygote viability and heterozygote fecundity.

GENOTYPE-DEPENDENT SPAWNING

In this section, we consider the possibility that spawning occurs at two discrete time intervals (union of gametes is at random within each interval) and that the amount of reproductive effort expended in each spawning period is genotype-dependent. However, the total reproductive effort per individual does not vary among genotypes. As before, the model is restricted to two alleles (A_1 and A_2) segregating at a single locus. First, we hypothesize that individuals expend reproductive effort (RE) according to the following scheme:

Genotype	A_1A_1	A_1A_2	A_2A_2
RE in first spawning period	$1 - k$	k	$1 - k$
RE in second spawning period	k	$1 - k$	k

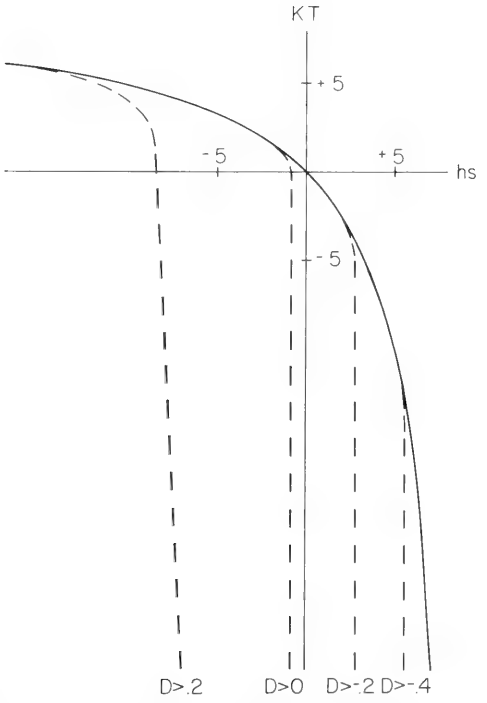

FIG. 3. Values of *hs* and *KT* which maintain a two-allele polymorphism (below solid line) and produce a heterozygote deficiency (*D*). The magnitude of *D* is indicated by the dashed lines. See text for explanation of the model.

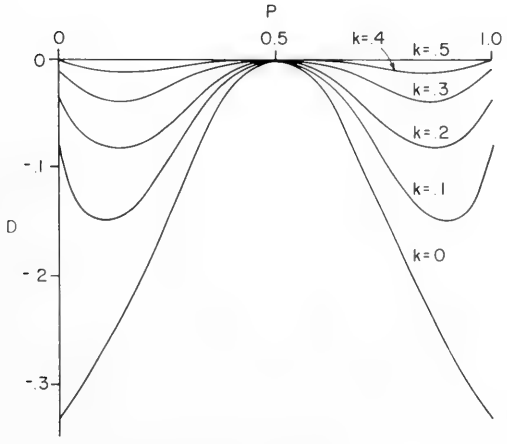

FIG. 4. Relationship between allele frequency (p) and heterozygote deficiency (D) for various values of overlap (k) under an overdominance model of genotype-dependent spawning time. See text for details.

where $0 \leq k \leq 0.5$. If spawning is "all or nothing," k may be thought of as the proportion of heterozygotes that spawn in the first period and the proportion of homozygotes that wait until the second period to spawn. Alternatively, k may be considered to be the proportion of total reproductive effort expended by each heterozygous individual in the first period and the corresponding proportion in the second period for each homozygous individual. In the present instance, the two conceptions give identical results. An explicit solution for D as a function of p (the frequency of allele A_1) and k appears to be unobtainable (Brian Golding, personal communication). However, we have obtained numerical results, which are presented in Fig. 4. First, we note that allele frequencies do not change over time. This result is not unexpected, because the spawning scheme presented above is simply a form of nonrandom mating, with no selective differences among genotypes. Second, we note that the magnitude of D is always less than or equal to 0, and is dependent on both p and k. The curves are symmetric around the line $p = 0.5$; furthermore, $D = 0$ whenever $p = 0.5$ or $k = 0.5$. However, in no case is the value of D less than -0.32. Furthermore, D values corresponding to those observed in the American oyster and other bivalves require skewed allele frequencies and also require no overlap between heterozygotes and homozygotes in spawning ($k = 0$).

Second, we consider the possibility that reproductive effort is expended according to a slightly different scheme:

Genotype	A_1A_1	A_1A_2	A_2A_2
RE in first spawning period	k	$1 - k$	$1 - k$
RE in second spawning period	$1 - k$	k	k

where $0 \leq k \leq 0.5$, as before. This model can be viewed as a "dominance" model for spawning time, as opposed to the previous one, which is an "overdominance" model. Again, we have been unable to obtain an explicit relationship between the value of D and the values of p and k. However, the numerical results are presented in Fig. 5. As before, allele frequencies do not change over time. Unlike the model presented earlier in this section, the curves are not symmetric around the line $p = q = 0.5$. Instead, the largest negative values of D are obtained when p is approximately equal to 0.72, for any

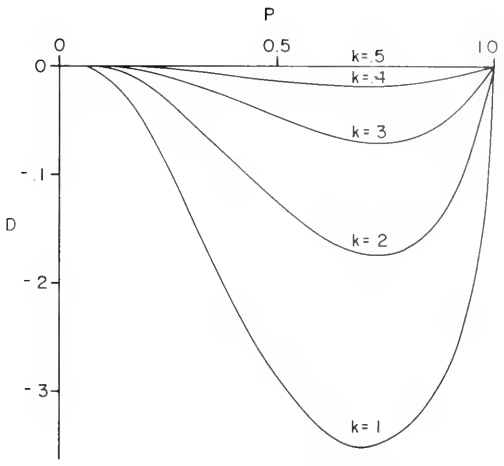

FIG. 5. Relationship between allele frequency (p) and heterozygote deficiency (D) for various values of overlap (k) under a dominance model of genotype-dependent spawning time. See text for details.

given k greater than 0. As the allele frequencies are skewed toward either 0 or 1, D approaches 0. Another important feature of this model is that when $k = 0$ (i.e. no overlap in spawning between A_1A_1 on one hand and A_1A_2 and A_2A_2 on the other), heterozygotes will always be lost from the population, regardless of the allele frequency. In this instance, the equilibrium value of D is -1 for $0 < p < 1$. By choosing values of k close to 0, we can generate deficiencies of any desired magnitude.

CONCLUSIONS

Although in this paper we have been concerned mostly with the numerical behavior of several models of population structure which might result in heterozygote deficiency, our choice of models was motivated by the biology and life history of bivalve molluscs. It is well-known that most bivalves exhibit both a planktonic larval stage and a sedentary adult stage, and it has been suggested (Koehn, 1975; Koehn *et al.*, 1976) that the various stages of the life cycle may be subject to different genetic forces. Our model of different pre-settlement and post-settlement viabilities was an attempt to determine if heterozygote inferiority at the pre-settlement stage could be compensated by heterozygote superiority at the adult stage. The model has two desirable properties. First, it predicts large deficiencies of heterozygotes in animals collected soon after settlement, which is consistent with the results of studies of several species of bivalves, such as *Mytilus californianus* (Tracey *et al.*, 1975) and *Crassostrea virginica* (Zouros *et al.*, 1983). Second, this model predicts that heterozygosity should increase in older (hence, larger) classes. As noted above, several studies of bivalve molluscs have reported that heterozygote deficiency is less marked in larger than in smaller size classes (see Wilkins, 1978, for a counter-example in the scallop *Pecten maximus*). Unfortunately, maintenance of large D values at settlement (less than -0.4) requires rather stringent conditions. In particular, the heterozygote must have a viability of less than 44% (compared to the homozygote standard) prior to settlement plus a relative viability of more than 220% after settlement. On the other hand, small but significant heterozygote deficiencies would not necessarily imply underdominance in larval viabilities; simple dominance of the allele with lower larval viability would suffice for this result.

To remove the burden of postulating large viability differences after settlement, and to account for the observation that for at least some loci (e.g. Lap in the American oyster) large heterozygote deficiencies may be observed even among older animals, we also considered a model in which adult animals experience genotype-dependent fertility differences, but viability differences are experienced only in the larval stage. Fertilization is external in most bivalve species, and each individual typically releases several million gametes. Thus, there is a potential for very large differences in fertility among genotypes. This model also maintains the polymorphism, and the results are very similar to the viability-viability model. Of course, the model cannot account for age- or size-dependent changes in heterozygosity after settlement. Both selection models require large selection differentials, a negative correlation between pre-settlement and post-settlement fitness components, and, for large heterozygote deficiencies, underdominance for larval viabilities. These requirements appear to be rather restrictive. One may, however, envision biological situations in which these conditions may obtain (for example, cross-fertilization of different species resulting in hybrids with low viability but high growth rates).

The models of genotype-dependent

spawning time were motivated by the observation that in *Crassostrea virginica* there appears to be a correlation between the degree of heterozygote deficiency and the degree of overdominance in growth rate among loci. Foltz *et al.* (1983) reported that in a one-year-old cohort of oysters collected as spat and grown under uniform conditions there were significant differences among genotypic classes in mean weight at all loci except Got-1 and Pgi, which are the two loci that do not exhibit marked heterozygote deficiencies (Zouros *et al.,* 1980). If the onset of spawning varies according to individual size (faster growing animals may reach sooner a critical gonad size or amount of glycogen reserve) then dependence of spawning time on weight will result in a genotype-dependent spawning time, inasuch as the phenotypic trait under consideration is genotypically determined. In a preliminary investigation, we have examined several more complex models in which spawning time is phenotype-dependent and overlap in spawning is proportional to the difference between genotypes in mean position on some quantitative scale such as body weight. The results indicate that loci exhibiting large differences in mean weight between genotypes would also exhibit large deficiencies of heterozygotes, everything else being constant. In this regard, we note that Wagener (1976) has developed a model which considers the effect of both mean and variance of a quantitative trait on mating preference.

Our results have implications for studies of natural populations of bivalve molluscs, in that they indicate the possible importance of several phenotypic characteristics (spawning time and growth rate) that have not been considered in most previous studies. In addition, our models might be applicable to other externally-fertilizing organisms that show heterozygote deficiency, such as some plants (e.g. *Liatris cylindracea*: Schaal & Levin, 1976), fishes (e.g. *Notropis stramineus*: Koehn *et al.,* 1971) and amphibians (e.g. *Bufo americanus*: Guttman & Wilson, 1973).

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REFERENCES CITED

AYALA, F. J., HEDGECOCK, D., ZUMWALT, G. S. & VALENTINE, J. W., 1973, Genetic variation in *Tridacna maxima,* an ecological analog of some unsuccessful evolutionary lineages. *Evolution,* 27: 177–191.

BEAUMONT, A. R., 1982, Variations in heterozygosity at two loci between year classes of a population of *Chlamys opercularis* (L.) from a Scottish sealoch. *Marine Biology Letters,* 3: 25–33.

BEAUMONT, A. R., DAY, T. R. & GAEDE, G., 1980, Genetic variation at the octopine dehydrogenase locus in the adductor muscle of *Cerastoderma edule* (L.) and six other bivalve species. *Marine Biology Letters,* 1: 137–148.

BOYER, J. F., 1974, Clinal and size-dependent variation at the *Lap* locus in *Mytilus edulis. Biological Bulletin,* 147: 535–549.

BUROKER, N. E., HERSHBERGER, W. K. & CHEW, K. K., 1975, Genetic variation in the Pacific oyster, *Crassostrea gigas. Journal of the Fisheries Research Board of Canada,* 32: 2471–2477.

BUROKER, N. E., HERSHBERGER, W. K. & CHEW, K. K., 1979a, Population genetics of the family Ostreidae. I. Intraspecific studies of *Crassostrea gigas* and *Saccostrea commercialis. Marine Biology,* 54: 157–169.

BUROKER, N. E., HERSHBERGER, W. K. & CHEW, K. K., 1979b, Population genetics of the family Ostreidae. II. Interspecific studies of the genera *Crassostrea* and *Saccostrea. Marine Biology,* 54: 171–184.

CHAISSON, R. E., SERUNIAN, L. A. & SCHOPF, T. J. M., 1976, Allozyme variation between two marshes and possible heterozygote superiority within a marsh in the bivalve *Modiolus demissus. Biological Bulletin,* 151: 404. Abstract.

COLGAN, D. J., 1981, Spatial and temporal variation in the genotypic frequencies of the mussel *Brachidontes rostratus. Heredity,* 46: 197–208.

FOLTZ, D. W., NEWKIRK, G. F. & ZOUROS, E., 1983, Genetics of growth rate in the American oyster: absence of interactions among enzyme loci. *Aquaculture,* 33: 157–165.

FUJIO, Y., 1979, Enzyme polymorphism and population structure of the Pacific oyster, *Crassostrea gigas. Tohoku Journal of Agricultural Research,* 30: 32–42.

GARTNER-KEPKAY, K. E., DICKIE, L. M., FREEMAN, K. R. & ZOUROS, E., 1980, Genetic differences and environments of mussel populations in the Maritime provinces. *Canadian Journal of Fisheries and Aquatic Sciences,* 37: 775–782.

GOSLING, E., 1982, Genetic variability in hatchery-produced Pacific oysters (*Crassostrea gigas* Thunberg). *Aquaculture,* 26: 273–287.

GOSLING, E. M. & WILKINS, N. P., 1981, Ecological genetics of the mussels *Mytilus edulis* and *M. galloprovincialis* on Irish coasts. *Marine Ecology—Progress Series,* 4: 221–227.

GROUE, K. J. & LESTER, L. J., 1982, A morphological and genetic analysis of geographic variation among oysters in the Gulf of Mexico. *Veliger,* 24: 331–335.

GUTTMAN, S. I. & WILSON, K. G., 1973, Genetic variation in the genus *Bufo.* I. An extreme degree of transferrin and albumin polymorphism in a population of the American toad (*Bufo americanus*). *Biochemical Genetics,* 8: 329–340.

HORNBACH, D. J., McLEOD, M. J., GUTTMAN, S. I. & SEILKOP, S. K., 1980, Genetic and morphological variation in the freshwater clam, *Sphaerium* (Bivalvia: Sphaeriidae). *Journal of Molluscan Studies,* 46: 158–170.

JOHNSON, A. G. & UTTER, F. M., 1973, Electrophoretic variants of aspartate aminotransferase of the bay mussel, *Mytilus edulis* (Linnaeus, 1758). *Comparative Biochemistry and Physiology,* 44B: 317–323.

KARTAVTSEV, Y. F., 1978, Genetic variability of a bivalve mollusk, the mussel *Crenomytilus grayanus* (Mytilidae). *Soviet Genetics,* 14: 186–191.

KOEHN, R. K., 1975, Migration and population structure in the pelagically dispersing marine invertebrate, *Mytilus edulis.* In: MARKERT, C. L., ed., *Isozymes, IV. Genetics and evolution.* Academic Press, New York, p. 945–959.

KOEHN, R. K., MILKMAN, R. & MITTON, J. B, 1976, Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel *Mytilus edulis. Evolution,* 30: 2–32.

KOEHN, R. K. & MITTON, J. B., 1972, Population genetics of marine pelecypods. I. Ecological heterogeneity and evolutionary strategy at an enzyme locus. *American Naturalist,* 106: 47–56.

KOEHN, R. K., PEREZ, J. E. & MERRITT, R. B., 1971, Esterase enzyme function and genetical structure of populations of the freshwater fish, *Notropis stramineus. American Naturalist,* 105: 51–69.

KOEHN, R. K., TURANO, F. J. & MITTON, J. B., 1973, Population genetics of marine pelecypods. II. Genetic differences in microhabitats of *Modiolus demissus. Evolution,* 27: 100–105.

LASSEN, H. H. & TURANO, F. J., 1978, Clinal variation and heterozygote deficit at the Lap locus in *Mytilus edulis. Marine Biology,* 49: 245–254.

LAVEE, D. & RITTE, U., 1977, Genetic parameters as indices of preadaptation for successful colonization in mytilids (Mollusca: Pelecypoda). *Israel Journal of Zoology,* 26: 259. Abstract.

LEVINTON, J. S. & FUNDILLER, D. L., 1975, An ecological and physiological approach to the study of biochemical polymorphisms. In: BARNES, H., ed., *Proceedings of the Ninth European Marine Biology Symposium.* Aberdeen University Press, Scotland, p. 165–178

MILKMAN, R. & BEATY, L. D., 1970, Large-scale electrophoretic studies of allelic variation in *Mytilus edulis. Biological Bulletin,* 139: 430. Abstract.

MILKMAN, R. & KOEHN, R. K., 1977, Temporal variation in the relationship between size, numbers, and an allele-frequency in a population of *Mytilus edulis. Evolution,* 31: 103–115.

MITTON, J. B., KOEHN, R. K. & PROUT, T., 1973, Population genetics of marine pelecypods. III. Epistasis between functionally related isoenzymes of *Mytilus edulis. Genetics,* 73: 487–496.

SCHAAL, B. A. & LEVIN, D. A., 1976, The demographic genetics of *Liatris cylindracea* Michx. (Compositae). *American Naturalist,* 110: 191–206.

SINGH, S. M. & ZOUROS, E., 1978, Genetic variation associated with growth rate in the American oyster (*Crassostrea virginica*). *Evolution,* 32: 342–353.

SKIBINSKI, D. O. F., AHMAD, M. & BEARDMORE, J. A., 1978, Genetic evidence for naturally occurring hybrids between *Mytilus edulis* and *Mytilus galloprovincialis. Evolution,* 32: 354–364.

SKIBINSKI, D. O. F. & BEARDMORE, J. A., 1979, A genetic study of intergradation between *Mytilus edulis* and *Mytilus galloprovincialis. Experientia,* 35: 1442–1444.

SKIBINSKI, D. O. F., BEARDMORE, J. A. & AHMAD, M., 1977, Genetic aids to the study of closely related taxa of the genus *Mytilus.* In: BATTAGLIA, B. & BEARDMORE, J. A., ed., *Marine organisms: genetics, ecology and evolution.* Plenum, New York, p. 469–486.

SKIBINSKI, D. O. F., McNEE, A. R. & BEARDMORE, J. A., 1978, Protein variation in the marine bivalve *Scrobicularia plana. Animal Blood Groups and Biochemical Genetics,* 9: 223–228.

TRACEY, M. L., BELLET, N. F. & GRAVEM, C. D., 1975, Excess allozyme homozygosity and breeding population structure in the mussel *Mytilus californianus. Marine Biology,* 32: 303–311.

WAGENER, D. K., 1976, Preferential mating. Nonrandom mating of a continuous phenotype. *Theoretical Population Biology,* 10: 185–204.

WILKINS, N. P., 1978, Length-correlated changes in heterozygosity at an enzyme locus in the scallop (*Pecten maximus* L.). *Animal Blood Groups and Biochemical Genetics,* 9: 69–77.

WILKINS, N. P. & MAHERS, N. F., 1973, Enzyme polymorphisms in the European oyster, *Ostrea edulis* L. *Animal Blood Groups and Biochemical Genetics,* 4: 41–47.

WILKINS, N. P. & MATHERS, N. F., 1974, Phenotypes of phosphoglucose isomerase in some bivalve molluscs. *Comparative Biochemistry and Physiology,* 48B: 599–611.

ZOUROS, E., SINGH, S. M. & MILES, H. E., 1980, Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution,* 34: 856–867.

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GENETIC DIVERSITY AND BREEDING SYSTEMS IN TERRESTRIAL SLUGS OF THE FAMILIES LIMACIDAE AND ARIONIDAE

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ABSTRACT

Genetic variation detected by electrophoresis of enzymes was surveyed in populations of 10 species of terrestrial slugs of the family Limacidae in Great Britain, Ireland and France, supplementing earlier studies of nine species of the family Arionidae in Europe and of introduced populations of species of both families in North America. Average individual heterozygosity varies widely across species, from zero in *Arion circumscriptus, A. fasciatus, A. silvaticus, A. intermedius* and *Deroceras agreste* to 0.19 in *A. distinctus* and *D. reticulatum.* The occurrence of monogenic (completely homozygous) strains of certain species is attributed to facultative or obligate self-fertilization, a breeding system that apparently is more widespread in the Arionidae than in the Limacidae. The genetic data indicate that outcrossing is the normal, if not exclusive, breeding system in most species of terrestrial slugs.

In terrestrial slugs in general, the breeding system has not been modified in the process of colonization of North America from Europe. But the breeding system is related to colonizing ability, with self-fertilizing European forms being disproportionately represented in North America. Among species, there is no apparent relationship between the breeding system and extent of geographic range or ecological amplitude. The only apparent ecological correlate of the breeding system is the fact that all the major pests, at least in Great Britain, are highly heterozygous outcrossing species.

Key words: breeding systems; electrophoresis; homozygosity; Mollusca; self-fertilization.

INTRODUCTION

Relationships between the genetic structure of populations and breeding systems are currently a major focus of research in evolutionary genetics. Much of the recent work on plants has been concerned with the extent and organization of genetic diversity in self-compatible species (Jain, 1976; Maynard Smith, 1978; Brown, 1979; Charlesworth & Charlesworth, 1979), while for animals the population genetics of parthenogenesis has been more intensively studied (e.g. Grassle & Shick, 1979). In the absence of adequate information on the extent and frequency of self-fertilization in animals, it has been widely assumed that selfing is rare, being employed only when there is no opportunity for outcrossing (Mather, 1973; Dobzhansky *et al.*, 1977; Roughgarden, 1979). This idea has persisted despite evidence that self-fertilization is the normal breeding system in several groups of animals (Thomas, 1959; Heard, 1965; Breder & Rosen, 1966; Birky, 1967; Cain, 1974; Harrington & Kallman, 1968; Hornback *et al.*, 1980)

Among malacologists, it is widely known from laboratory studies that many species of hermaphroditic snails and slugs are capable of reproducing uniparentally (reviews in Runham & Hunter, 1970; Duncan, 1975). For several species, breeding experiments employing genetic markers have demonstrated that self-fertilization (or, possibly, automictic parthenogenesis) rather than apomictic (non-recombinational) parthenogenesis is involved (Ikeda, 1937; Williamson, 1959), but in most cases selfing has merely been presumed because parthenogenesis apparently is rare in molluscs (Runham & Hunter, 1970; Solem, 1974; Duncan, 1975; Fretter & Peake, 1978). Until recently, the pulmonate land snail *Rumina decollata* (Linnaeus, 1758) was the only mollusc in which the regular occurrence of selfing in natural populations had been demonstrated (Selander & Kaufman, 1973; Selander & Hudson, 1976; Selander & Ochman, 1983).

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However, it has now been determined that there is a variety of breeding systems in terrestrial slugs (Mollusca: Pulmonata), ranging from obligate outcrossing to frequent, and, apparently, in some cases, obligate self-fertilization (McCracken & Selander, 1980). Thus, terrestrial slugs would seem to provide particularly favorable material for comparative studies of the distribution and evolution of breeding systems.

The population genetic studies of terrestrial slugs by McCracken & Selander (1980) were based on 14 species sampled in the eastern United States, most of which were introduced from Europe. Objectives of our more recent research have been (1) to determine whether the breeding systems of the native European populations of the various species are the same as those of introduced North American populations; (2) to extend the study of population structure and breeding systems to species not represented in North America; and (3) to identify possible ecological correlates of variation in the breeding system. In the present study, genetic variation and population structure were analyzed in seven species of the family Limacidae (*Milax sowerbyi, M. budapestensis, Limax maximus, L. pseudoflavus, L. marginatus, Deroceras caruanae,* and *D. reticulatum*), as represented by populations in Great Britain, Ireland, and France. Additionally, we have had available the results of studies of *Milax gagates, Limax tenellus,* and *Deroceras agreste* by Leslie R. Noble (unpublished data, 1981). Comparable data for nine species of the family Arionidae are presented elsewhere (Foltz *et al.,* 1982b). Conclusions suggested by our analyses of genetic diversity and breeding systems in both the Limacidae and Arionidae are summarized in the Discussion of the present paper.

MATERIALS AND METHODS

Samples were collected in gardens, on roadsides, and in other disturbed sites at 24 localities in Great Britain, 26 in Ireland, and three in France (Table 1), in areas less than 50 m^2. Specimens were identified to species

TABLE 1. Collection localities (counties in parentheses).

England
1 Kinver (Stafford)
2 Botanic Gardens, Birmingham (Warwick)
3 King's Heath (Warwick)
4 Edgbaston (Warwick)
5 Stadhampton (Oxford)
6 Lower Woodend (Buckingham)
8 Merton (London)
9 Ewell (London)
10 Faccombe (Hampshire)
11 Houghton (Hampshire)
12 Fordingbridge (Hampshire)
13 Edmondsham (Dorset)
14 Sandford (Dorset)
15 Chickerell (Dorset)
17 Colyton (Devon)
18 Umberleigh (Devon)
20 Rackenford (Devon)
21 Minehead (Somerset)
22 Nettlecombe Park, Williton (Somerset)
23 Nettlecombe Park, Williton (Somerset)
24 Southway (Somerset)

Wales
26 Carmarthen (Carmarthen)
27 Red Roses (Carmarthen)
28 3 km S Pembroke (Pembroke)

Ireland
30 Gardiner Street (Dublin)
31 Opposite St. Patrick's Cathedral (Dublin)
32 Kilmeadan (Waterford)
33 Cork (Cork)
34 Summer Cove (Cork)
35 1 km W Clonakilty (Cork)
36 2 km W Leap (Cork)
37 2 km N Bantry (Cork)
38 2 km SW Glengariff (Cork)
39 Glanmore Lough (Kerry)
40 Killarney (Kerry)
41 Castleisland (Kerry)
42 Nenagh (Tipperary)
43 2 km SE Hurlers Cross (Clare)
44 Spanish Point (Clare)
45 Inagh (Clare)
48 Ennistymon (Clare)
49 5 km E Ennistymon (Clare)
56 Cashel (Galway)
62 Aillebrack Lough (Galway)
65 Kill (Galway)
66 Streamstown (Galway)
69 Letterfrack (Galway)
74 Kylemore Lough (Galway)
75 2 km W Leenaun (Galway)
78 1.5 km N Westport (Mayo)

France
81 Orsay (Essonne)
82 St. Michel (Essonne)
83 Lardy (Essonne)

by morphological characters (Kerney & Cameron, 1979),[2] and then processed for electrophoresis by methods described by McCracken & Selander (1980).

In many cases, such as for the *Arion hortensis, A. fasciatus*, and *Limax flavus* complexes, specimens were identified by comparing their electrophoretic profiles to those of standards kindly provided by Stella Davies, R. A. D. Cameron or Leslie R. Noble.

Procedures for horizontal starch-gel electrophoresis (Electrostarch, lot 307) and enzyme staining are outlined by Selander *et al.* (1971) and Harris & Hopkinson (1976). From 11 to 16 of the following enzymes were scored in each species: α-glycerophosphate dehydrogenase (α-*Gpd*), several malate dehydrogenases (*Mdh*), indophenol oxidase (*Ipo*), glutamic oxaloacetic transaminase (*Got*), several phosphoglucomutases (*Pgm*), several esterases (*Est*), several peptidases (*Pep*), several leucine aminopeptidases (*Lap*), mannosephosphate isomerase (*Mpi*), and phosphoglucose isomerase (*Pgi*). The preferred buffer systems were lithium hydroxide for *Est, Pep,* and *Lap*; continuous tris-citrate I for α-*Gpd, Mdh, Mpi,* and *Pgi*; and continuous tris-citrate II for *Ipo, Got* and *Pgm*. Isoenzymes coded by separate loci were numbered in order of decreasing electrophoretic mobility, but the same numbers do not necessarily indicate homology of proteins among species. Except as noted below, electromorphs were equated with alleles, which for each polymorphic locus were designated alphabetically, in order of decreasing mobility of their electromorphs. Again, a particular letter does not necessarily refer to the same allele in different species.

Allele frequencies were determined by direct count, except for a null allele (absence of enzyme activity) at *Pgm-1* in *Limax marginatus,* the frequency of which was estimated by the method of Schull (1965). The frequency of the slower- (or slowest-) migrating electromorph at each locus has been omitted from the tables. Observed heterozygosity per locus per individual in a sample (H_o) was determined by direct count, and expected heterozygosity (H_e) was calculated from allele frequencies, with correction for small sample size (Levene, 1949). Mean heterozygosity for a species (H_o) was estimated by averaging values for all samples. For each polymorphic locus, a fixation index (F_{ID}) was calculated as $F_{ID} = 1 - H_o/H_e$, and these values, weighted by expected numbers of heterozygotes, were averaged over samples to obtain a mean fixation index ($\bar{F}_{ID}$). Other *F*-statistics for each locus were obtained by Wright's (1978) method of hierarchical analysis, which adjusts for sampling variance. Three levels of population structure were recognized: demes (sample localities) within regions (F_{DR}); regions within subdivisions (F_{RS}); and subdivisions within the total area sampled (F_{ST}) (Single, isolated localities were not included in these analyses.) An *F*-value of zero indicates that there was no added variance component at that level.

RESULTS

Milax sowerbyi (Férussac, 1823)

A total of 63 individuals was collected at three localities in England and three sites in Ireland (Table 2). Seven of the 16 loci examined were polymorphic, and observed heterozygosity (H_o) was high in all samples, with a mean of 0.126.

For the analysis of *F*-statistics (Table 2), the localities were assigned to two subdivisions: England (localities 9, 12 and 14) and Ireland (66 and 74). There was considerable differentiation of demes within subdivisions, as reflected in the high values of F_{DS}. The unusual heterogeneity within subdivisions shown by *Pep* is caused by the fixation of different alleles in the two Irish samples. In contrast, there was relatively little heterogeneity between subdivisions ($\bar{F}_{ST}$ = 0.058). For four loci, there was no added variance component at this level, but allele frequencies at *Lap-2* were significantly heterogeneous, largely because of the absence of *Lap-2*^{b} in the two Irish samples.

The mean fixation index ($\bar{F}_{ID}$) for seven loci showed considerable scatter, as expected in small samples. However, the absence of a consistent departure from Hardy-Weinberg proportions indicates that *M. sowerbyi* reproduces predominantly, if not entirely, by outcrossing.

Milax budapestensis (Hazay, 1881)

Although this species has been introduced throughout the British Isles (Kerney & Camer-

[2]According to Dr. Howard Ochman (letter dated December 3, 1982) voucher specimens for this study are filed under accession number 2314 with the Department of Molluscs at the British Museum (Natural History), London.

TABLE 2. *Milax sowerbyi*: allele frequencies and *F*-statistics for α-glycerophosphate dehydrogenase (α-*Gpd*), phosphoglucomutase-2 (*Pgm-2*), esterase-2 (*Est-2*), esterase-3 (*Est-3*), esterase-4 (*Est-4*), peptidase (*Pep*), and leucine aminopeptidase-2 (*Lap-2*). N = sample size, H_o = observed heterozygosity.

Locality	N	H_o	α-*Gpd*[a]	*Pgm-2*[a]	*Pgm-2*[b]	*Est-2*[a]	*Est-2*[b]	*Est-3*[a]	*Est-3*[b]	*Est-4*[a]	*Pep*[a]	*Lap-2*[a]
9	10	0.14	0.75	1.00	0.00	0.45	0.55	0.55	0.45	0.80	0.95	0.55
12	14	0.13	0.43	0.96	0.04	0.21	0.07	0.32	0.68	0.96	0.50	0.57
14	8	0.19	0.19	0.87	0.13	0.44	0.00	0.81	0.19	0.31	0.69	0.50
40	7	0.13	0.57	1.00	0.00	0.29	0.00	1.00	0.00	0.64	1.00	0.64
66	8	0.06	0.00	0.94	0.06	0.69	0.00	0.94	0.00	0.75	1.00	1.00
74	16	0.11	0.22	0.91	0.06	0.25	0.00	1.00	0.00	0.56	0.00	1.00

Statistic	Mean	α-*Gpd*	*Pgm-2*	*Est-2*	*Est-3*	*Est-4*	*Pep*	*Lap-2*
F_{ID}	0.036	0.088	−0.037	−0.148	0.340	−0.184	−0.007	0.201
F_{DS}	0.243	0.255	0.003	0.310	0.194	0.294	0.646	0.000
F_{ST}	0.058	0.021	0.000	0.000	0.134	0.000	0.000	0.252

Monomorphic loci: *Mdh-1, Mdh-2, Ipo, Got, Pgm-1, Est-1, Lap-1, Mpi, Pgi.*

TABLE 3. *Milax budapestensis:* allele frequencies and *F*-statistics for malate dehydrogenase-1 (*Mdh-1*), esterase-2 (*Est-2*), peptidase-2 (*Pep-2*), leucine aminopeptidase-1 (*Lap-1*), and leucine aminopeptidase-2 (*Lap-2*).

Locality	N	H_o	*Mdh-1*[a]	*Est-2*[a]	*Est-2*[b]	*Pep-2*[a]	*Lap-1*[a]	*Lap-2*[a]	*Lap-2*[b]
30	20	0.17	0.58	0.25	0.05	0.35	0.72	0.35	0.03
31	10	0.11	0.50	0.55	0.06	0.00	0.80	0.00	0.00
32	11	0.07	1.00	0.73	0.00	0.00	0.45	0.00	0.09
34	13	0.14	0.85	0.84	0.08	0.31	0.54	0.42	0.00
38	15	0.10	0.70	0.60	0.00	0.23	1.00	0.20	0.00
41	9	0.16	0.94	0.67	0.27	0.28	0.83	0.28	0.00
78	20	0.07	1.00	0.88	0.00	0.05	0.85	0.05	0.50

Statistic	Mean	*Mdh-1*	*Est-2*	*Pep-2*	*Lap-1*	*Lap-2*
F_{ID}	0.084	0.148	0.050	−0.060	0.246	0.037
F_{DR}	0.101	0.022	0.115	0.082	0.166	0.120
F_{RS}	0.024	0.078	0.043	0.000	0.000	0.000

Monomorphic loci: *Mdh-2, Ipo, Got, Pgm, Est-1, Pep-1, Mpi, Pgi.*

on, 1979), we found it in appreciable numbers only in Ireland. Five of 13 loci examined were polymorphic, and mean heterozygosity was 0.117 (Table 3).

For the *F*-statistics analysis, localities were assigned to two regions in Ireland: Dublin (localities 30 and 31) and southwestern Ireland (34, 38, and 41). The F_{DR} values, which are reasonably uniform across loci, reflect considerable local heterogeneity, but there was only a small added component of variance between regions (F_{RS}) at each locus.

The mean fixation indices reflect a strong tendency toward heterozygote deficiency, but the deviation from Hardy-Weinberg equilibrium proportions of genotypes is not uniform over loci. The minor departure from Hardy-Weinberg proportions at *Est-2, Pep-2,* and *Lap-2* can be attributed to random sampling error; but the larger deviations at *Mdh-1* and *Lap-1* are more problematical, and are perhaps caused by undetected null alleles or by the lumping of individuals from different demes into the same samples. Whatever the actual causes of the heterogeneity among loci, the lack of a consistent level of heterozygote deficiency across loci would seem to exclude the possibility of frequent self-fertilization.

TABLE 4. *Limax maximus*: allele frequencies for leucine aminopeptidase-1 (*Lap-1*).

Locality	N	H_o	*Lap-1*[a]
1	23	0.01	0.87
4	7	0.02	0.86
5	13	0.04	0.73
65	10	0.03	0.70

Monomorphic loci: α-*Gpd, Mdh, Ipo, Got, Pgm-1, Pgm-2, Est-1, Est-2, Pep, Lap-2, Mpi, Pgi.*

Limax maximus Linnaeus, 1758

Only one of the 13 loci assayed in this species was polymorphic (Table 4). Mean heterozygosity was 0.027, and the mean fixation index was −0.005. The value of F_{DS}, calculated from the three samples from England (1, 4 and 5), was 0.022. On the basis of this information, populations of *L. maximus* appear to be locally panmictic and relatively homogeneous spatially.

Limax tenellus Müller, 1774

According to an analysis of British material by Leslie R. Noble (in preparation), this species is an outcrosser which, like *L. maximus* in Europe, carries a relatively low level of heterozygosity (see Table 10).

Limax pseudoflavus Evans, 1978

This species, long confused with *L. flavus* (Evans, 1978), was collected at two localities in Ireland: 12 specimens at Aillebrack Lough (62) and 11 specimens at Kill (65). Genetic variation was limited to weak polymorphisms at two of the 14 loci examined: At *Pep-2* and *Lap-2*, variant alleles at a frequency of 0.05 were detected in the sample from Kill. The monomorphic loci were α-*Gpd, Mdh, Ipo, Got, Pgm-1, Pgm-2, Est-1* and *Est-2, Pep-1, Lap-1, Mpi* and *Pgi.*

Mean heterozygosity was only 0.007. Because of the limited information available, the nature of the breeding system is unclear.

Limax marginatus Müller, 1774

In this species, four of 13 loci were polymorphic, but heterozygosity was generally low, with a mean of 0.034 (Table 5). *Pgm-1* was polymorphic for four alleles, one of which, *Pgm-1*^{o}, was a null. Its presence in sample 17 was indicated by the occurrence of one specimen lacking a band (electromorph) for this protein. Even when this specimen was excluded from the sample, a marked deficiency of heterozygotes remained ($P < 0.005$).

For the hierarchical analysis, two subdivisions were designated: southwestern England (17 and 24) and western Ireland (69 and 75). Substantial heterogeneity among demes within subdivisions (F_{DS}) was shown by *Pgm-1* and *Est-3*, but there was little or no variation at *Got* and *Pgm-2*. In contrast to the local heterogeneity at two loci, there was no added component of variance for any locus at the subdivision level.

Inasmuch as the mean fixation indices, based on all samples, showed a slight tendency toward heterozygote excess, there

TABLE 5. *Limax marginatus*: allele frequencies and *F*-statistics for glutamic oxaloacetic transaminase (*Got*), phosphoglucomutase-1 (*Pgm-1*), phosphoglucomutase-2 (*Pgm-2*) and esterase-3 (*Est-3*).

Locality	N	H_o	*Got*[a]	*Pgm-1*^{o*}	*Pgm-1*[a]	*Pgm-1*[b]	*Pgm-2*[a]	*Est-3*[a]
17	12	0.06	1.00	0.37	0.25	0.38	0.08	1.00
24	13	0.01	1.00	0.00	0.00	1.00	0.04	1.00
40	13	0.04	0.85	0.00	0.00	0.96	0.00	0.92
69	11	0.01	0.95	0.00	0.00	1.00	0.00	1.00
75	6	0.05	1.00	0.00	0.00	1.00	0.00	0.50

Statistic	Mean	*Got*	*Pgm-1*	*Pgm-2*	*Est-3*
F_{ID}	−0.073	−0.106	0.000	−0.030	−0.156
F_{DS}	0.229	0.007	0.449	0.000	0.458
F_{ST}	0.006	0.000	0.000	0.024	0.000

Monomorphic loci: α-*Gpd, Mdh, Ipo, Est-1, Est-2, Pep, Lap, Mpi, Pgi.*
*Null allele.

TABLE 6. *Deroceras caruanae*: allele frequencies and *F*-statistics for α-glycerophosphate dehydrogenase (α-*Gpd*), esterase-1 (*Est-1*) and leucine aminopeptidase-2 (*Lap-2*).

Locality	N	H_o	α-*Gpd*[a]	*Est-1*[a]	*Lap-2*[a]	*Lap-2*[b]
4	9	0.12	0.44	0.50	0.00	0.17
9	15	0.08	0.20	0.50	0.10	0.23
17	16	0.02	0.00	0.25	0.00	0.00
20	10	0.06	0.05	0.80	0.00	0.25
26	13	0.09	0.12	0.65	0.00	0.31
30	12	0.03	0.12	0.79	0.00	0.00
31	10	0.03	0.00	0.60	0.00	0.00
36	10	0.05	0.20	0.75	0.00	0.00
37	14	0.07	0.25	0.39	0.04	0.00
38	8	0.07	0.12	0.75	0.00	0.06
40	12	0.03	0.04	0.71	0.00	0.00
42	8	0.04	0.06	0.69	0.00	0.00
48	8	0.05	0.25	0.69	0.00	0.00
49	23	0.04	0.00	0.59	0.00	0.00
56	9	0.03	0.00	0.61	0.00	0.00
69	14	0.05	0.14	0.43	0.00	0.00
78	17	0.01	0.00	0.88	0.00	0.00
81	16	0.03	0.00	0.25	0.00	0.00
83	20	0.04	0.00	0.47	0.03	0.00

Statistic	Mean	α-*Gpd*	*Est-1*	*Lap-2*
F_{ID}	0.071	0.045	0.125	0.042
F_{DR}	0.078	0.070	0.109	0.055
F_{RS}	0.024	0.071	0.000	0.000
F_{ST}	0.045	0.000	0.041	0.094

Monomorphic loci: *Mdh-1, Mdh-2, Ipo, Got, Pgm, Est-2, Pep, Lap-1, Mpi, Pgi.*

is no reason to believe that the breeding system of this species involves anything but outcrossing.

Deroceras caruanae (Pollonera, 1891)

Thirteen loci were examined, three of which were polymorphic (Table 6). Mean heterozygosity was 0.049 (range, 0.01–0.12).

For the *F*-statistics analysis, the samples were assigned to three subdivisions. (a) England, consisting of two regions, each of which included two samples (4, 9, and 17, 20, respectively). (b) Ireland, consisting of four regions: Dublin (30 and 31), southwestern Ireland (36, 37, 38 and 40), western Ireland (42, 48 and 49), and northwestern Ireland (56, 69 and 78). (c) Central France, including two samples (81 and 83).

Substantial heterogeneity among demes within regions was reflected by the uniform and relatively high values of F_{DR}; but there was little heterogeneity among regions and among subdivisions. Only the F_{ST} value for *Lap-2* was large.

The mean fixation indices, based on 19 samples, showed a deficiency of heterozygotes at all three polymorphic loci. For α-*Gpd* and *Lap-2*, the number of samples showing heterozygote deficiency was balanced by an almost equal number of samples with heterozygote excess. Only three of 19 samples demonstrated a consistent departure from Hardy-Weinberg equilibrium at all loci. The large heterozygote deficiency at *Est-1* (Table 6) suggests the presence of a null allele, since no other locus shows a deficiency of this magnitude. In view of this possibility and the moderate size of the deviations from Hardy-Weinberg proportions at α-*Gpd* and *Lap-2*, we conclude that the predominant, if not exclusive, mode of reproduction is outcrossing.

Deroceras agreste (Linnaeus, 1758)

In collections from many localities in Scandinavia, Leslie R. Noble (unpublished data, 1981) found no heterozygosity in any individual. Several loci were polymorphic,

TABLE 7. *Deroceras reticulatum*: allele frequencies for α-glycerophosphate dehydrogenase (*α-Gpd*), glutamic oxaloacetic transaminase (*Got*), phosphoglucomutase-1 (*Pgm-1*), and phosphoglucomutase-2 (*Pgm-2*).

Locality	N	H_o	*α-Gpd*^{a}	*α-Gpd*^{b}	*Got*^{a}	*Pgm-1*^{a}	*Pgm-1*^{b}	*Pgm-1*^{c}	*Pgm-2*^{a}	*Pgm-2*^{b}	*Pgm-2*^{c}
1	17	0.21	0.12	0.85	0.00	0.12	0.53	0.32	0.00	1.00	0.00
2	27	0.22	0.35	0.63	0.00	0.00	0.93	0.07	0.06	0.92	0.02
3	22	0.24	0.20	0.73	0.09	0.11	0.75	0.09	0.00	1.00	0.00
4	12	0.18	0.38	0.62	0.00	0.00	0.88	0.12	0.04	0.96	0.00
5	27	0.23	0.33	0.67	0.00	0.00	0.39	0.59	0.04	0.92	0.04
6	25	0.18	0.20	0.80	0.00	0.06	0.69	0.21	0.00	0.90	0.08
8	19	0.22	0.11	0.89	0.00	0.00	0.66	0.31	0.00	0.92	0.08
9	24	0.24	0.18	0.82	0.02	0.10	0.63	0.19	0.00	0.93	0.07
10	25	0.15	0.14	0.86	0.02	0.04	0.92	0.04	0.04	0.90	0.04
11	23	0.19	0.02	0.98	0.00	0.04	0.70	0.00	0.00	0.94	0.04
12	13	0.23	0.08	0.92	0.04	0.11	0.35	0.04	0.00	1.00	0.00
13	27	0.23	0.21	0.75	0.09	0.02	0.48	0.05	0.00	1.00	0.00
15	18*	0.13	0.15	0.73	0.00	0.00	0.28	0.03	0.00	0.97	0.03
17	25	0.18	0.10	0.88	0.00	0.21	0.23	0.42	0.00	0.79	0.21
18	12	0.18	0.21	0.79	0.00	0.12	0.50	0.38	0.00	1.00	0.00
20	16	0.24	0.31	0.66	0.00	0.03	0.66	0.28	0.00	0.94	0.00
21	12	0.21	0.29	0.71	0.00	0.04	0.58	0.38	0.04	0.96	0.00
22	25	0.19	0.15	0.85	0.00	0.02	0.74	0.24	0.04	0.94	0.02
23	24	0.19	0.10	0.88	0.00	0.04	0.60	0.34	0.00	0.98	0.00
24	29	0.18	0.12	0.88	0.00	0.12	0.81	0.07	0.00	1.00	0.00
27	29	0.22	0.33	0.67	0.09	0.05	0.95	0.00	0.19	0.81	0.00
28	11	0.13	0.27	0.73	0.00	0.04	0.96	0.00	0.00	0.95	0.05
32	15	0.18	0.30	0.70	0.00	0.07	0.93	0.00	0.00	1.00	0.00
33	18	0.14	0.03	0.94	0.00	0.08	0.92	0.00	0.00	1.00	0.00
34	16	0.16	0.12	0.88	0.00	0.06	0.94	0.00	0.00	1.00	0.00
35	10	0.15	0.11	0.89	0.00	0.00	1.00	0.00	0.00	1.00	0.00
36	10	0.16	0.20	0.80	0.00	0.00	1.00	0.00	0.00	0.94	0.06
37	18	0.17	0.08	0.92	0.03	0.00	1.00	0.00	0.00	1.00	0.00
39	24	0.08	0.06	0.94	0.00	0.00	1.00	0.00	0.00	1.00	0.00
41	25	0.19	0.04	0.92	0.10	0.06	0.94	0.00	0.00	1.00	0.00
42	10	0.27	0.06	0.94	0.00	0.32	0.68	0.00	0.00	1.00	0.00
43	10	0.25	0.19	0.81	0.00	0.00	0.90	0.10	0.00	1.00	0.00
44	21	0.21	0.42	0.58	0.00	0.02	0.87	0.11	0.00	1.00	0.00
45	10	0.24	0.20	0.80	0.00	0.05	0.90	0.05	0.00	1.00	0.00
66	10	0.16	0.62	0.38	0.00	0.00	1.00	0.00	0.00	1.00	0.00
81	10	0.16	0.10	0.90	0.00	0.00	0.30	0.70	0.00	1.00	0.00
82	15†	0.19	0.09	0.91	0.00	0.13	0.37	0.50	0.00	0.96	0.00

Monomorphic loci: *Mdh, Ipo, Est-2.*
*15 for *Pgm-2.*
†11 for *α-Gpd.*

however, so that a number of strains, differing, for the most part, at single loci, can be distinguished. This species shares many alleles with *D. reticulatum,* with which it has frequently been confused because of close similarity in external morphology. Our data indicate that *D. agreste* is self-fertilizing, and this conclusion is supported by reports (Luther, 1915; Maury & Reygrobellet, 1963) that it has been maintained for many generations in the laboratory by uniparental reproduction.

Deroceras reticulatum (Müller, 1774)

Eleven loci were examined, of which eight were polymorphic (Tables 7 and 8). Heterozygosity was high (0.192) and fairly consistent across samples. Populations on the southwestern coast of Ireland had relatively low heterozygosity (0.14), but no other patterns were apparent.

For the *F*-statistics analysis (Table 9), the samples were arranged in three subdivisions. (a) Great Britain, consisting of five regions:

TABLE 8. *Deroceras reticulatum:* allele frequencies for esterase-1 (*Est-1*), leucine aminopeptidase (*Lap*), mannose phosphate isomerase (*Mpi*), and phosphoglucose isomerase (*Pgi*).

Sample	$Est\text{-}1^a$	$Est\text{-}1^b$	$Est\text{-}1^c$	$Est\text{-}1^d$	Lap^b	Lap^c	Mpi^a	Mpi^b	Mpi^c	Pgi^a	Pgi^b	Pgi^c
1	0.00	0.26	0.62	0.12	0.00	1.00	0.00	0.71	0.29	0.15	0.00	0.53
2	0.00	0.18	0.65	0.15	0.06	0.94	0.07	0.65	0.28	0.26	0.00	0.57
3	0.00	0.32	0.43	0.25	0.02	0.98	0.09	0.89	0.02	0.34	0.04	0.55
4	0.00	0.25	0.54	0.21	0.00	1.00	0.04	0.83	0.13	0.13	0.00	0.54
5	0.00	0.09	0.80	0.09	0.00	1.00	0.00	0.81	0.19	0.11	0.00	0.54
6	0.00	0.15	0.83	0.02	0.00	1.00	0.00	0.80	0.20	0.13	0.00	0.52
8	0.00	0.24	0.74	0.02	0.05	0.95	0.10	0.66	0.24	0.24	0.00	0.55
9	0.02	0.17	0.75	0.02	0.04	0.96	0.02	0.69	0.29	0.02	0.00	0.60
10	0.06	0.06	0.78	0.10	0.02	0.98	0.00	0.90	0.10	0.16	0.00	0.52
11	0.02	0.13	0.77	0.08	0.02	0.98	0.00	0.73	0.27	0.14	0.02	0.40
12	0.00	0.19	0.69	0.12	0.00	1.00	0.08	0.81	0.11	0.35	0.00	0.50
13	0.00	0.14	0.79	0.07	0.02	0.96*	0.05	0.93	0.02	0.26	0.02	0.36
15	0.00	0.03	0.92	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.83
17	0.00	0.00	0.94	0.06	0.00	1.00	0.12	0.74	0.14	0.00	0.02	0.48
18	0.00	0.12	0.88	0.00	0.00	1.00	0.08	0.71	0.17**	0.04	0.00	0.46
20	0.00	0.13	0.84	0.03	0.00	1.00	0.09	0.72	0.19	0.00	0.00	0.34
21	0.00	0.46	0.54	0.00	0.04	0.96	0.21	0.79	0.00	0.08	0.05	0.08
22	0.00	0.30	0.66	0.02	0.06	0.92	0.00	0.92	0.08	0.08	0.00	0.30
23	0.00	0.28	0.60	0.10	0.12	0.88	0.02	0.94	0.04	0.08	0.00	0.22
24	0.00	0.20	0.65	0.15	0.10	0.83	0.07	0.79	0.14	0.18	0.00	0.47
27	0.00	0.48	0.31	0.17	0.00	1.00	0.03	0.81	0.14†	0.00	0.07	0.72
28	0.00	0.21	0.79	0.00	0.00	1.00	0.00	1.00	0.00	0.04	0.00	0.58
32	0.00	0.07	0.86	0.07	0.17	0.83	0.07	0.93	0.00	0.10	0.00	0.33
33	0.00	0.03	0.80	0.17	0.06	0.91†	0.08	0.92	0.00	0.06	0.06	0.66
34	0.00	0.00	0.81	0.19	0.00	1.00	0.19	0.81	0.00	0.24	0.00	0.33
35	0.00	0.00	0.80	0.20	0.35	0.65	0.17	0.83	0.00	0.20	0.15	0.15
36	0.00	0.05	0.85	0.10	0.10	0.90	0.00	1.00	0.00	0.11	0.11	0.39
37	0.00	0.05	0.92	0.03	0.42	0.58	0.06	0.80	0.14	0.25	0.03	0.30
39	0.00	0.00	0.81	0.19	0.15	0.85	0.00	1.00	0.00	0.00	0.06	0.86
41	0.00	0.00	0.76	0.24	0.18	0.82	0.28	0.64	0.08	0.40	0.02	0.08
42	0.00	0.09	0.68	0.23	0.14	0.86	0.15	0.80	0.05	0.55	0.00	0.05
43	0.00	0.25	0.65	0.10	0.70	0.30	0.05	0.95	0.00	0.39	0.00	0.22
44	0.00	0.26	0.63	0.11	0.41	0.59	0.07	0.93	0.00	0.63	0.00	0.30
45	0.00	0.15	0.80	0.05	0.30	0.70	0.17	0.83	0.00	0.30	0.00	0.55
66	0.00	0.00	0.70	0.30	0.20	0.80	0.10	0.90	0.00	0.05	0.10	0.80
81	0.05	0.45	0.40	0.10	0.00	1.00	0.00	0.85	0.15	0.00	0.00	0.90
82	0.00	0.27	0.66	0.07	0.00	0.97	0.00	0.86	0.14	0.00	0.00	0.80

*0.02 for Lap^a.
**0.04 for Mpi^e.
†0.02 for Mpi^d.
‡0.03 for Lap^a.

Birmingham (1–4); London (5–6 and 8–11); south-central England (12–15); southwestern England (18 and 20–24); and southern Wales (27 and 28). (b) Ireland, consisting of two regions: southern coast (32–37 and 39) and western region (41–45 and 66). (c) France, consisting of two samples (81 and 82). Heterogeneity among demes ($\bar{F}_{DR}$) was moderate; but less variation was observed at the higher levels, although values of $\bar{F}_{RS}$ were large for *Pgm-1, Est-1* and *Pgi.* Only *Lap-2* showed a large added variance component among subdivisions.

TABLE 9. *Deroceras reticulatum*: hierarchical *F*-statistics for eight polymorphic loci.

Locus	$\bar{F}_{ID}$	$\bar{F}_{DR}$	$\bar{F}_{RS}$	$\bar{F}_{ST}$
α-Gpd	−0.051	0.067	0.010	0.000
Got	−0.065	0.048	0.004	0.000
Pgm-1	−0.008	0.093	0.125	0.055
Pgm-2	0.031	0.034	0.017	0.002
Est-1	0.074	0.032	0.038	0.004
Lap	0.070	0.128	0.005	0.104
Mpi	−0.028	0.040	0.010	0.008
Pgi	0.013	0.097	0.048	0.002
Grand mean	0.005	0.067	0.032	0.022

Fixation indices displayed no consistent pattern, and the grand mean was negligible (0.005), which is compatible with the conclusion that outcrossing is the normal, if not exclusive, breeding system in this species.

DISCUSSION

An earlier analysis of genetic variation in species of the family Arionidae revealed that populations of the species of *Arion* introduced to North America are similar in both genetic structure and breeding system to native populations of the same species in Britain (Foltz *et al.,* 1982b). Of the seven species of the Limacidae examined in the present study, only *Limax maximus* and *Deroceras reticulatum* are widely established in North America and were included in the study by McCracken & Selander (1980). The genetic evidence indicates that outcrossing is the predominant (and probably exclusive) mode of reproduction in both the native and introduced populations. Thus, for terrestrial slugs in general there is no evidence that the breeding system has been modified in the process of colonization of North America from Europe. There does, however, appear to be a relationship between colonizing ability and breeding system. Among the native European species of arionids and limacids that have become firmly established and widespread in eastern North America, self-fertilizing forms are disproportionately represented. Thus, of the eight self-fertilizing species so far identified in the British fauna, only one (*Deroceras agreste*) has failed to colonize eastern North America. In contrast, nine of 13 outcrossing British species are not represented in eastern North America. (This difference is significant, $p = .03$, by Fisher's exact test.)

The greater success of selfing species of terrestrial slugs in colonizing is paralleled in plants, in which a similar association has long been recognized. Historically, self-compatibility in plants and animals has been interpreted largely in terms of ecological or demographic models (Baker, 1965; Stebbins, 1957, 1958; Tomlinson, 1966; Ghiselin, 1969; Grant, 1975), centering on the reproductive assurance that selfing provides in the initial stages of colonization, together with a possible "infective principle" (Stebbins, 1958)—the ability of a successful selfing genotype to multiply more rapidly than an outcrosser in a new environment. A similar demographic interpretation can be invoked for terrestrial molluscs as well. This is certainly the most parsimonious explanation for our findings, and whether it will eventually be necessary to invoke genetical hypotheses remains to be determined. Among these are Baker's (1965, 1972) concept of the selectional development of general purpose genotypes in good colonizers and their maintenance by selfing or apomixis; and Solbrig's (1976) consideration of the cost-benefit trade-off of outcrossing and selfing when mating involves closely related individuals in small, inbred colonies.

Related to the problem of the relationship of the breeding system to colonization is the more fundamental question of the relationship of genetic variation within populations to ecological amplitude. Is there any advantage to one mating system or another (and the resultant genetic structure of the population) in a given environment? As noted by Roughgarden (1979) and Bell (1982), no unequivocal answer is available, notwithstanding that it has been a primary concern of ecological genetics for many years. Several recent reviews of this subject have reached the conclusion that there is serious reason to question the intuitively appealing and widespread notion (e.g. Nevo, 1978) that the niche width or ecological amplitude of a population or species is a reflection of the genetic diversity among individuals in the population (Hedrick *et al.,* 1976; Mitter & Futuyma, 1979; Schnell & Selander, 1981). A particularly significant recent finding is that of Mitter and Futuyma (1979), who discovered that in geometrid moths polyphagous species are in fact less polymorphic than are monophagous species.

Information on terrestrial slugs that is relevant to this problem is very limited, but perhaps sufficient to support the conclusion that outcrossing species having large amounts of polymorphism and heterozygosity do not have wider geographical ranges or occupy a greater diversity of habitats than do those forms consisting of one or a few monogenic strains generated by self-fertilization. Thus, the geographical ranges of self-fertilizing slugs in western Europe do not appear to be more restricted, on the average, than those of outcrossing species (Kerney & Cameron, 1979). Additionally, our own field experience and the distribution records compiled by Chichester & Getz (1969, 1973) and Getz & Chichester (1971) suggest that selfing species in eastern North America do not occupy

smaller ranges than do the outcrossing species. There is also no evidence of a consistent difference in niche breadth between selfing and outcrossing species, although few quantitative ecological data are available. In an ecological survey near Ithaca, New York, Beyer & Saari (1977, 1978) found that *Arion fasciatus* and *A. subfuscus* (almost certainly the selfing form) occur in a wider range of habitats and are more common (particularly in native vegetation) than are the outcrossing species *Deroceras reticulatum* and *Philomycus carolinianus.* And on an experimental flat of woodland near Ithaca, niche breadth for 7 species of slugs was measured in terms of 88 physical, chemical, and floristic features by the method of Colwell & Futuyma (1971) (McCracken & Selander, 1980). Ranked in decreasing order of niche breadth, the species were *A. fasciatus, A. circumscriptus, A. subfuscus* (selfing form), *D. reticulatum, D. laeve, A. intermedius* and *A. silvaticus.* Thus, three species that consist of one or a few monogenic strains had broader niches than did the outcrossing and highly polymorphic *D. reticulatum.* Finally, we may cite Jennings & Barkham's (1975) study of foraging height above ground for individuals of four European species. For the two selfing species studied, *A. fasciatus* and *A. intermedius*, coefficients of variation in foraging height were 2.53 and 2.62, respectively, while for the outcrossing species, *D. reticulatum* and *A. hortensis,* the corresponding values were 1.14 and 2.42. Incidentally, on the English woodlot where their study was conducted, the two selfing species were the most abundant, together comprising 72% of the total slug population.

An observation that is perhaps contrary to the above conclusion is that, at least in Great Britain, all of the species of terrestrial slugs that are major agricultural pests are highly heterozygous outcrossing species (Barnes & Weil, 1944, 1945; Bett, 1960; Hunter, 1966; Stephenson, 1968), whereas the less heterozygous species are not common in agricultural areas. For six species (*Arion subfuscus, A. hortensis, A. distinctus, Milax sowerbyi, M. budapestensis,* and *Deroceras reticulatum*) which are well-known agricultural pests in Britain and continental Europe, the mean heterozygosity across species (with standard error) is 0.121 ± 0.025. (We have assumed that most records of *A. subfuscus* in agricultural habitats refer to the outcrossing form.) For the 13 non-pest species included in Table 10 (for Europe), the corresponding figure is significantly lower: 0.026 ±0.007.

Inasmuch as none of the eight species that are exclusive or partial selfers is a pest, whereas 6 of 13 outcrossers are major pests, there seems to be a relationship with breeding system. Whether this is a reflection of greater adaptability of outcrossing species to novel habitat conditions warrants further investigation. The greater heterozygosity of agricultural pests may be a consequence of population mixing by transportation of soils and agricultural products, but we doubt that this explanation is alone sufficient to explain the observed relationship.

Nicklas & Hoffmann (1981) presented the results of breeding experiments on *D. laeve,* which they interpreted as evidence for reproduction by apomictic parthenogenesis. However, that explanation is incompatible with the low level of heterozygosity reported for this species by McCracken & Selander (1980), by Foltz *et al.* (1982a), and by Nicklas & Hoffmann themselves. Hoffmann (1983) later modified their interpretation to admit the possibility of automictic parthenogenesis. Theoretical studies of parthenogenesis by Asher (1970) and others have shown that automictic parthenogenesis can "mimic" the effects of either apomictic parthenogenesis or self-fertilization, depending on the mechanism for restoration of diploidy and the amount of recombination at the marker locus under investigation. Therefore, even breeding studies of the sort employed by Nicklas & Hoffmann (1981) may not yield unambiguous results. Instead, complete elucidation of the breeding system in *D. laeve* and other slugs must await cytological investigation. McCracken & Selander (1980) and Foltz *et al.* (1982b) recognized that an automictic mode of reproduction other than self-fertilization could explain the paucity or absence of heterozygosity in some species of terrestrial slugs. However, this uncertainty does not affect the conclusions of this study, which are: (1) that there is a diversity of genetic structures among terrestrial slugs; (2) that some species are not locally panmictic; and (3) that the same pattern has occurred independently in two distantly-related families of slugs.

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TABLE 10. Population structure and breeding system in 22 species of terrestrial slugs

Family	Species	North America		Europe		Breeding system
		H_o	P	H_o	P	
Arionidae	*Arion ater ater*			0	0	Mixed[1,4]
	A. ater rufus			.059	.31	Outcrossing[1,4,7]
	A. lusitanicus			.082	.25	Outcrossing[4]
	A. subfuscus (A)	.197	.64	.062	.15	Outcrossing[2,4]
	A. subfuscus (B)			0	0	Mixed[4]
	A. fasciatus	0	0			Self-fertilizing[2]
	A. circumscriptus	0	0	0	0	Self-fertilizing[2,4]
	A. silvaticus	0	0	0	.07	Self-fertilizing[2,4]
	A. hortensis	.126	.42	.041	.14	Outcrossing[4,6]
	A. distinctus	.175	.67	.186	.38	Outcrossing[4,6]
	A. intermedius	0	.20	0	.20	Self-fertilizing[2,4]
	A. owenii			.044	.23	Outcrossing[4]
Limacidae	*Milax sowerbyi*			.126	.44	Outcrossing[5]
	M. budapestensis			.117	.39	Outcrossing[5]
	M. gagates			.013	.08	Outcrossing[7]
	Limax maximus	.166	.45	.027	.08	Outcrossing[2,5]
	L. tenellus			.028	.14	Outcrossing[7]
	L. pseudoflavus			.007	.14	Outcrossing (?)[5]
	L. marginatus			.034	.31	Outcrossing[5]
	L. valentianus	.077	.33			Outcrossing[2]
	Deroceras laeve	.005	.78			Mixed[3]
	D. caruanae			.049	.23	Outcrossing (?)[5]
	D. agreste			0	.29	Self-fertilizing[7]
	D. reticulatum	.188	.80	.192	.73	Outcrossing[2,5]

Sources:
[1]Burnet (1972).
[2]McCracken & Selander (1980).
[3]Foltz *et al.* (1982a).
[4]Foltz *et al.* (1982b).
[5]This study.
[6]Selander & McCracken, unpublished data, 1979.
[7]Noble, unpublished data, 1981.

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REFERENCES CITED

ASHER, J. H., Jr., 1970, Parthenogenesis and genetic variability. II. One-locus models for various diploid populations. *Genetics*, 66: 369–391.

BAKER, H. G., 1965, Characteristics and modes of origin of weeds. In: BAKER, H. G. & STEBBINS, G. L., ed., *The Genetics of Colonizing Species*. Academic Press, New York, p. 147–168.

BAKER, H. G., 1972, Migrations of weeds. In: VALENTINE, D. H., ed., *Taxonomy, Phytogeography and Evolution*. Academic Press, New York, p. 327–347.

BARNES, H. F. & WEIL, J. W., 1944, Slugs in gardens: their numbers, activities and distribution. Part 1. *Journal of Animal Ecology*, 13: 140–175.

BARNES, H. F. & WEIL, J. W., 1945, Slugs in gardens: their numbers, activities and distribution. Part 2. *Journal of Animal Ecology*, 14: 71–105.

BELL, G., 1982, *The Masterpiece of Nature. The Evolution and Genetics of Sexuality*. University of California Press, Berkeley, 635 p.

BETT, J. A., 1960, The breeding seasons of slugs in gardens. *Proceedings of the Zoological Society of London*, 135: 559–568.

BEYER, W. N. & SAARI, D. M., 1977, Effect of tree species on the distribution of slugs. *Journal of Animal Ecology*, 46: 697–702.

BEYER, W. N. & SAARI, D. M., 1978, Activity and ecological distribution of the slug *Arion subfuscus* (Draparnaud) (Stylommatophora, Arionidae). *American Midland Naturalist,* 100: 359–367.

BIRKY, C. W., Jr., 1967, Studies on the physiology and genetics of the rotifer, *Asplanchna. Journal of Experimental Zoology,* 164: 105–116.

BREDER, C. M., Jr. & ROSEN, D. E., 1966, *Modes of Reproduction in Fishes.* Natural History Press, Garden City, New York, 941 p.

BROWN, A. H. D., 1979, Enzyme polymorphism in plant populations. *Theoretical Population Biology,* 15: 1–42.

BURNET, B., 1972, Enzyme protein polymorphism in the slug *Arion ater. Genetical Research,* 20: 161–173.

CAIN, A. J., 1974, Breeding system of a sessile animal. *Nature,* 247: 289–290.

CHARLESWORTH, D. & CHARLESWORTH, B., 1979, The evolutionary genetics of sexual systems in flowering plants. *Proceedings of the Royal Society of London,* B 205: 513–530.

CHICHESTER, L. F. & GETZ, L. L., 1969, The zoogeography and ecology of arionid and limacid slugs introduced into northeastern North America. *Malacologia,* 7: 313–346.

CHICHESTER, L. F. & GETZ, L. L., 1973, The terrestrial slugs of northeastern North America. *Sterkiana,* 51: 11–42.

COLWELL, R. K. & FUTUYMA, D. J., 1971, On the measurement of niche breadth and overlap. *Ecology,* 52: 567–576.

DOBZHANSKY, T., AYALA, F. J., STEBBINS, G. L. & VALENTINE, J. W., 1977, *Evolution.* Freeman, San Francisco, 572 p.

DUNCAN, C. J., 1975, Reproduction. In: FRETTER, V. & PEAKE, J., ed., *Pulmonates, Vol. 1. Functional Anatomy and Physiology.* Academic Press, London, p. 309–366.

EVANS, N. J., 1978, *Limax pseudoflavus* sp. n., a new species of slug for Ireland. *Irish Naturalists Journal,* 19: 173.

FOLTZ, D. W., SCHAITKIN, B. M. & SELANDER, R. K., 1982a, Gametic disequilibrium in the self-fertilizing slug *Deroceras laeve. Evolution,* 36: 80–85.

FOLTZ, D. W., OCHMAN, H., JONES, J. S., EVANGELISTI, S. M. & SELANDER, R. K., 1928b, Genetic population structure and breeding systems in arionid slugs (Mollusca: Pulmonata). *Biological Journal of the Linnean Society,* 17: 225–241.

FRETTER, V. & PEAKE, J., ed., 1978, *Pulmonates, Vol. 2A. Systematics, Evolution, and Ecology.* Academic Press, London, xi + 540 p.

GETZ, L. L. & CHICHESTER, L. F., 1971, Introduced European slugs. *The Biologist,* 53: 118–127.

GHISELIN, M. T., 1969, The evolution of hermaphroditism among animals. *Quarterly Review of Biology,* 44: 189–208.

GRANT, V., 1975, *Genetics of Flowering Plants.* Columbia University Press, New York, 514 p.

GRASSLE, J. F. & SHICK, J. M., ed., 1979, Symposium: ecology and asexual reproduction in animals. *American Zoologist,* 19: 667–797.

HARRINGTON, R. W., Jr. & KALLMAN, K. D., 1968, The homozygosity of clones of the self-fertilizing hermaphroditic fish *Rivulus marmoratus* Poey (Cyprinodontidae, Atheriniformes). *American Naturalist,* 102: 337–343.

HARRIS, H. & HOPKINSON, D. A., 1976, *Handbook of Enzyme Electrophoresis in Human Genetics.* North-Holland, Amsterdam. No pagination.

HEARD, W. H., 1965, Comparative life histories of North American pill clams (Sphaeriidae: *Pisidium*). *Malacologia,* 2: 381–411.

HEDRICK, P. W., GINEVAN, M. E. & EWING, E. P., 1976, Genetic polymorphism in heterogeneous environments. *Annual Review of Ecology and Systematics,* 7: 1–32.

HOFFMANN, R. J., 1983, The mating system of the terrestrial slug *Deroceras laeve. Evolution,* 37: 423–425.

HORNBACH, D. J., McLEOD, M. J., GUTTMAN, S. I. & SEILKOP, S. K., 1980, Genetic and morphological variation in the freshwater clam, *Sphaerium* (Bivalvia: Sphaeriidae). *Journal of Molluscan Studies,* 46: 158–170.

HUNTER, P. J., 1966, The distribution and abundance of slugs on an arable plot in Northumberland. *Journal of Animal Ecology,* 35: 543–557.

IKEDA, K., 1937, Cytogenetic studies on the self-fertilization of *Philomycus bilineatus* Benson. *Journal of Science of Hiroshima University, Series B, Division 1 (Zoology),* 5: 67–123.

JAIN, S. K., 1976, The evolution of inbreeding in plants. *Annual Review of Ecology and Systematics,* 7: 469–495.

JENNINGS, T. J. & BARKHAM, J. P., 1975, Slug populations in mixed deciduous woodland. *Oecologia,* 20: 279–286.

KERNEY, M. P. & CAMERON, R. A. D., 1979, *A Field Guide to the Land Snails of Britain and North-west Europe.* Collins, London, 288 p.

LEVENE, H., 1949, On a matching problem arising in genetics. *Annals of Mathematical Statistics,* 20: 91–94.

LUTHER, A., 1915, Zuchtversuche an ackerschnecken (*Agriolimax reticulatus* Mull. und *Agr. agrestis* L.). *Acta Societatis pro Fauna et Flora Fennica,* 40: 1–42.

MATHER, K., 1973, *Genetical Structure of Populations.* Chapman and Hall, London, 197 p.

MAURY, M.-F. & REYGROBELLET, D., 1963, Sur les distinctions specifiques chez les mollusques Limacides du genre *Deroceras. Comptes Rendus de l'Académie des Sciences Paris,* 257: 276–277.

MAYNARD SMITH, J., 1978, *The Evolution of Sex.* Cambridge University Press, Cambridge, 222 p.

McCRACKEN, G. F. & SELANDER, R. K, 1980, Self-fertilization and monogenic strains in natural populations of terrestrial slugs. *Proceedings of the National Academy of Sciences U.S.A.,* 77: 684–688.

MITTER, C. & FUTUYMA, D. J., 1979, Population genetic consequences of feeding habits in some forest Lepidoptera. *Genetics*, 92: 1005–1021.

NEVO, E., 1978, Genetic variation in natural populations: patterns and theory. *Theoretical Population Biology*, 13: 121–177.

NICKLAS, N. L. & HOFFMANN, R. J., 1981, Apomictic parthenogenesis in a hermaphroditic terrestrial slug, *Deroceras laeve* (Müller). *Biological Bulletin*, 160: 123–135.

ROUGHGARDEN, J., 1979, *Theory of Population Genetics and Evolutionary Ecology: An Introduction*. Macmillan, New York, 634 p.

RUNHAM, N. W. & HUNTER, P. J., 1970, *Terrestrial Slugs*. Hutchinson, London, 184 p.

SCHNELL, G. D. & SELANDER, R. K., 1981, Environmental and morphological correlates of genetic variation in mammals. In: SMITH, M. H. & J. JOULE, ed., *Mammalian Population Genetics*. University of Georgia Press, Athens, p. 60–99.

SCHULL, W. J., 1965, Estimation of genetic parameters in population studies. In: NEEL, J. V., SHAW, M. W. & SCHULL, W. J., ed., *Genetics and the Epidemiology of Chronic Diseases*. U.S. Public Health Service, Washington, p. 45–60.

SELANDER, R. K. & HUDSON, R. O., 1976, Animal population structure under close inbreeding: the land snail *Rumina* in southern France. *American Naturalist*, 110: 695–718.

SELANDER, R. K. & KAUFMAN, D. W., 1973, Self-fertilization and genetic population structure in a colonizing land snail. *Proceedings of the National Academy of Sciences U.S.A.*, 70: 1186–1190.

SELANDER, R. K. & OCHMAN, H., 1983, The genetic structure of populations as illustrated by molluscs. In: RATTAZZI, M. C., SCANDALIOS, J. C. & WHITT, G. S., ed., *Isozymes: Current Topics in Biological and Medical Research*, Vol. 10, p. 92–103. Liss, New York.

SELANDER, R. K., SMITH, M. H., YANG, S. Y., JOHNSON, W. E. & GENTRY, J. B., 1971, Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). *Studies in Genetics*, 6: 49–90.

SOLBRIG, O. T., 1976, On the relative advantages of cross- and self-fertilization. *Annals of the Missouri Botanical Gardens*, 63: 262–276.

SOLEM, G. A., 1974, *The Shell Makers: Introducing Mollusks*. Wiley, New York, 289 p.

STEBBINS, G. L., 1957, Self-fertilization and population variability in the higher plants. *American Naturalist*, 91: 337–354.

STEBBINS, G. L., 1958, Longevity, habitat, and release of genetic variability in the higher plants. *Cold Spring Harbor Symposia on Quantitative Biology*, 23: 365–378.

STEPHENSON, J. W., 1968, A review of the biology and ecology of slugs of agricultural importance. *Proceedings of the Malacological Society of London*, 38: 169–178.

THOMAS, G. J., 1959, Self-fertilization and production of young in a sphaeriid clam. *Nautilus*, 72: 131–140.

TOMLINSON, J., 1966, The advantages of hermaphroditism and parthenogenesis. *Journal of Theoretical Biology*, 11: 54–58.

WILLIAMSON, M., 1959, Studies on the colour and genetics of the black slug. *Proceedings of the Royal Physical Society of Edinburgh*, 27: 87–93.

WRIGHT, S., 1978, *Evolution and the Genetics of Populations. Vol. 4, Variability Within and Among Natural Populations*. University of Chicago Press, Chicago, 580 p.

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USE OF MOLECULAR GENETICS TO DISTINGUISH SPECIES OF THE GASTROPOD GENUS *CREPIDULA* (PROSOBRANCHIA: CALYPTRAEIDAE)

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ABSTRACT

Populations of *Crepidula convexa* and *C. plana* were collected in mangroves near Ft. Pierce, Florida. They were found to differ from New England populations in mode of larval development, although this character is not known to vary intraspecifically in the genus. Allozyme studies were conducted to assess the genetic differences between Floridian and northern populations of these taxa. Horizontal starch gel electrophoresis resolved 24 loci for about 50 individuals of each population. Three populations of the Floridian *C. convexa* and two of *C. plana* were compared with three each of supposedly the same species from New England. To assess the typical amount of genetic difference within and between species, seven populations of *C. fornicata* and one each of Californian *C. onyx* and Brasilian *C. protea* were electrophoresed.

The populations of *C. fornicata* clustered tightly, with Nei's distance values (D) of 0.003–0.016. The three Floridian *C. convexa* were separated by D = 0.008 – 0.076, while the separation of three populations of northern *C. convexa* was 0.037 – 0.057. The two groups coalesced at D = 0.764, using a simple unweighted averaging technique. For southern and northern *C. plana*, D values were 0.045 and 0.052 – 0.097, respectively; the groups coalesced at D = 0.393. *C. convexa*'s greater difference between regions is due to the lack of shared alleles at 50% of the loci. *C. plana* is characterized more by large differences in allele frequencies; only 17% of the loci lack shared alleles. Calculations based on Rogers' distance gave similar results with higher cophenetic correlations.

These data demonstrate lack of gene exchange between the Floridian and northern populations of both species, especially *C. convexa*. Because the populations are allopatric, electrophoretic data alone cannot conclusively delineate species. D values over 0.30 are strong indicators that speciation has occurred, according to data from other molluscan taxa. In this study, divergence between known species of *Crepidula* (e.g., *C. fornicata* and *C. plana*) was greater than that between members of either of the potential sibling species pairs being tested.

Crepidula "convexa" from Florida produces pediveliger young, whereas northern *C.convexa* release metamorphosed, crawling young. *C. "plana"* from Ft. Pierce releases crawling young, whereas northern *C. plana* releases veligers. These differences, coupled with the electrophoretic patterns, call for future anatomical comparisons that will most probably lead to recognition of these taxa as new species.

Key words: *Crepidula plana; Crepidula convexa;* Florida; molecular genetics; electrophoresis; cryptic species; larval development.

INTRODUCTION

Three recognized species of the marine gastropod genus *Crepidula* are common along the entire East Coast of the United States. They have been the subjects of studies in embryology (Conklin, 1897), morphology (Werner, 1951, 1955), physiology (Krüger, 1970), life history (Coe, 1936; Hoagland, 1978), pollution and laboratory rearing (Calabrese & Rhodes, 1974). Despite this attention, systematics of the genus remains problematic. Species determination has been based almost entirely on shell characters (e.g. Berry, 1950), although there are few discrete shell characters, and shell phenotype often varies with the substrate. Larval development and body pigment patterns provide additional valuable species characters (Hoagland, 1977), but are not known for many species. One new species, *C. fecunda,* was initially discovered via a difference in larval development (Gallardo, 1977, 1979).

The white, flat species of *Crepidula,* sometimes placed in the subgenus *Ianacus* Mörch, 1852, are particularly difficult to treat systematically. In the Eastern Pacific, two types are known: species that release planktonic larvae

after a short brooded period and those that brood larvae through metamorphosis. In the Atlantic Ocean and adjacent bodies of water, three species all belonging to the former type are known: *C. unguiformis* Lamarck of the Mediterranean Sea, *C. plana* Say of North America, and *C. protea* d'Orbigny of South America (Hoagland, 1983). I predicted from the deployment of such sympatric species pairs in California and Panama that there could be ecological space in the subtropical and tropical Atlantic for another white, flat species (Hoagland, 1975, 1976) that released crawling young instead of veligers. If such a species were found, it should be smaller than the species with planktonic larvae, as is true of most other *Crepidula* (Hoagland, 1978).

Another problem in the systematics of *Crepidula* involves a second North American species, *Crepidula convexa* Say, which had been thought to be distributed from Nova Scotia to the Caribbean. Museum collections show divergent shell color patterns: white with brown spots or streaks in populations from Florida and the Caribbean, and purple or brown with red lines in Northern populations. In Virginia and North Carolina, shells of both types have been found, but not in the same immediate locality. One character such as shell color is an insufficient basis for a belief that more than one species exists, especially since the populations are allopatric. *C. convexa* in Florida often lives on species of *Cerithium* with color patterns similar to itself and unlike the more solid, darker color of substrates in New England such as *Littorina littorea.*

I collected supposed *C. convexa* and *C. plana* from Florida to compare with northern populations of the two species. This paper reports data from an analysis of 24 allozyme loci using starch-gel electrophoresis, and tests the hypothesis that these two forms of Floridian *Crepidula* are genetically and possibly specifically distinct from the more northern Atlantic populations. The third widely-distributed Atlantic species, *C. fornicata* (Linnaeus), is used as a reference species, as are a species from the Pacific, *C. onyx* Sowerby, and *C. protea* from Brasil. Two other species, *C. aculeata* (Gmelin) from Miami, Florida, and *C. lingulata* Gould from Balboa Island, California, are included only for a general summary of unique alleles, because less than 50 individuals have been electrophoresed. The genetic data are used to construct indices of relatedness among the taxa, including the amount of genetic differentiation within and between species. The results are considered in the light of the literature on genetic distances.

Information on the life history and larval development of the two Floridian taxa is also reported and compared with similar information for the northern *C. plana* and *C. convexa.* The degree of geographical isolation of populations of these taxa along the Atlantic Coast of North America is assessed. Conclusions as to the general usefulness of molecular and life historical data for taxonomic assessment of allopatric populations are drawn.

METHODS

Species Examined and Localities

Crepidula fornicata was collected by hand at low tide in 0–0.5 m of water from six localities in New England (Table 1); one population, at Bridgeport, Connecticut, was sampled in both 1980 and 1981. *Crepidula protea* was dredged off Rio Grande do Sul, Brasil, and *C. onyx* and *C.lingulata* were collected at low

TABLE 1. Populations studied.

Species	Population	Habitat description	Latitude; longitude	ANSP voucher #
C. fornicata	Bridgeport, Conn. (Br)	Estuary, St. Mary's by the Sea, on shells. In stacks	41°10′N; 73°10′W	354548 and *353353*
	Marine Biol. Lab. Beach, Woods Hole, Mass. (MBL)	Base of rock jetties, sand beach, on stones	41°32′N; 70°39′W	354547
	Woods Hole Yacht Club, Woods Hole, Mass. (WH)	Yacht basin, muddy sand substrate, on shells and bottles	41°32′N; 70°39′W	355372

TABLE 1 (Continued)

Species	Population	Habitat description	Latitude; longitude	ANSP voucher #
	Millstone Point, Niantic Bay, Conn. (M)	Bay, muddy sand substrate with rocks, on scallops and other shells. In stacks	41 18'N; 72 9'W	(A 8691) 353352
	Little Compton, R.I. (LC)	Estuary, on shells. In stacks	41 31'N; 71 10'W	355337
	Kettle Cove, Maine (K)	Lower tide pools, on rocks	43 33'N; 70 13'W	354549
C. onyx	Balboa Is., Calif. (B)	Ocean side, on mussels at base of pilings, subtidal. In stacks	33 30'N; 117 55'W	355340
C. protea	Rio Grande do Sul, Brasil (RG)	Dredged, 20–30 m, on shell rubble	32 30'S; 52 00'W	355341
C. convexa	Millstone, Conn. (M)	As *C. fornicata* (no stacks)	41 18'N; 72 9'W	353351
	Bridgeport, Conn. (Br)	As *C. fornicata* (no stacks)	41°10'N; 73°10'W	355338
	Little Compton, R.I. (LC)	Estuary, near mouth, on shells	41 31'N; 71°10'W	355373
	Little Jim Creek, Indian River, near Ft. Pierce Inlet, Fla. (LJC)	Mangroves, on shells and bottles	27°28'N; 80°18'30"W	(A 9395) 357839 & 355370
	Blue Hole, Indian River, near Ft. Pierce Inlet, Fla. (BH)	Mangroves, on shells and bottles	27°32'N; 80°20'W	(A 9396) 355335
	Sabine Is., Gulf Breeze, Fla. (GB)	In oyster tables, E.P.A. lab.	30°20'N; 87°5'W	(A 9397) 355344
C. plana	Bridgeport, Conn. (Br)	As *C. convexa*	41°10'N; 73°10'W	355336
	Millstone, Conn. (M)	As *C. convexa*	41°18'N; 72°9'W	353350
	Little Compton, R.I. (LC)	As *C. convexa*	41°31'N; 71°10'W	354550
	Little Jim Creek, Ft. Pierce, Fla. (LJC)	As *C. convexa*	27°28'N; 80°18'30"W	357838 & 355371
	Blue Hole, Ft. Pierce, Fla. (BH)	As *C. convexa*	27°32'N; 80°20'W	(A 9394) 355334
C. aculeata	Key Biscayne, Biscayne Bay, Fla.	On *Pinna* and *Strombus*, 0.5–1.0 m	25°40'N; 80°12'W	355342
	Ft. Pierce Inlet, Fla.	On bridge supports (cement & boulders)	27°32'N; 80°20'W	355343
C. lingulata	Balboa Is., Calif.	On mussels at base of pilings, subtidal	33°30'N; 117°55'W	355340

tide, 0–0.5 m, from Balboa Island, California (Table 1). A minimum of 50 individuals per population was collected and frozen in tris tissue buffer (pH 7.4) until electrophoresed. Exact sample sizes are given in the Appendix.

Specimens of *Crepidula plana* and *C. convexa* were collected from three northern localities and frozen for later study (Table 1). Both were found intertidally on stones, shells, and old bottles, although *C. plana* was most often inside shells and bottles. About 10 specimens of *C. plana* were collected at Biscayne Bay, near Miami, Florida, but most died before they could be electrophoresed. Observations were made on the type of larvae they carried. Specimens referable to *C. plana* and *C. convexa* as well as a few of *C. aculeata* were collected in mangrove and oyster reef areas on hard substrates in two adjacent areas of the Indian River near the Ft. Pierce Inlet, east coast of Florida, at a depth of 0.5 m. The two areas were separated by a channel and sand bars on which no specimens were found. The Ft. Pierce specimens were returned alive on their natural substrates to the laboratory, where they were sexed, after which the number and developmental stage of embryos for each brooding female were recorded. Adults were then frozen in tris tissue buffer. Some broods were retained with the female, undisturbed, and allowed to hatch in salt-water-filled finger bowls; observations on developmental stage at hatching were made. Specimens similar to the *C. convexa* of Ft. Pierce were received live from the Environmental Protection Agency (E.P.A.) laboratory at Gulf Breeze, west coast of Florida. They had grown in oyster tanks in the laboratory. They were sexed and their larvae were observed prior to electrophoresis of adults.

Some adults of each species were fixed in formalin and preserved in 70% ethyl alcohol as voucher specimens. All shells of electrophoresed specimens were also catalogued. These specimens are housed in the Academy of Natural Sciences; ANSP numbers are given in Table 1.

Electrophoresis

Horizontal starch-gel electrophoresis was carried out, followed by staining for 19 specific enzymes. The general methods of Ayala *et al.* (1973) as amended by Dillon & Davis (1980) and Davis *et al.* (1981) were applied to these mollusks. Starch gels (13%) were pre-

TABLE 2. Enzymes assayed, buffers, current, voltage, and duration of electrophoresis.

Enzyme	No. loci	Gel & tray buffer	Current/ voltage	Run time (hr)
Acid phosphatase (AcPh)	1	TC6	35 MA	3.0
Adenylate kinase (Adkin)	1	Poulik	35 MA	3.0
Aldehyde oxidase (AO)	1	TEB 9	350 V	4.5
Aspartate amino transferase (AAT)	1	TEB 9	350 V	4.5
Esterase NA (EST NA)	2	TEB 9/8	35 MA	2.0
Glucose-6-phosphate dehydrogenase (G6PDH)	1	Poulik	35 MA	3.0
Glucose-phosphate isomerase (GPI = PGI)	1	TC 6	35 MA	2.0
Hexokinase (HEX)	1	TEB 8	35 MA	3.5
Lactate dehydrogenase (LDH)	1	TEB 9/8	35 MA	2.0
Leucine amino peptidase (LAP)	2	TC 6	35 MA	2.0
Mannose-6-phosphate isomerase (MPI)	2	Poulik	35 MA	3.0
NAD-dependent malate dehydrogenase (NAD-MDH)	2	TC 6	35 MA	3.0
Peptidase G (Pep G)	1	TEB 8	35 MA	3.5
Peptidase T (Pep T)	2	TEB 8	35 MA	3.5
Phosphoglucomutase (PGM)	1	TEB 9/8	35 MA	2.0
		TC 6	35 MA	2.0
6-phosphogluconate dehydrogenase (6PGDH)	1	TEB 8	35 MA	3.5
Sorbitol dehydrogenase (SoDH)	1	TEB 9	350 V	4.5
Superoxide dismutase (SOD)	1	TEB 9/8	35 MA	2.0
Xanthine dehydrogenase (XDH)	1	Poulik	35 MA	3.0
	24			

pared using 33.5 g of Electrostarch and 250 ml of one of four gel buffers: 1) tris citrate, pH 6.0; 2) tris NaOH borate (Poulik), tray buffer pH 7.6 and gel buffer pH 8.9; 3) tris-EDTA-borate (TEB) pH 8.0; and 4) TEB, pH 9.1. Five enzyme systems were run on TEB of pH 9.1 but with tray buffer of pH 8. Table 2 reports the gel and tray buffers, current, and run time for each of the enzymes.

Six wicks of No. 3 Whatman filter paper were saturated with homogenized tissue from each individual. They were blotted and applied, one wick from each individual, to each of six gels to be run concurrently. The gels were sliced into three or four slabs for staining, so a maximum of 24 enzyme systems could be examined in one day.

Because 31 wicks fit on one gel, 25 individuals from the population being tested plus up to six individuals from a reference population could be run on a single gel. Runs were repeated if the relative position of the reference population was unclear or if results were otherwise ambiguous. A sample size of at least 50 individuals per population was sought for each enzyme locus, requiring each population to be run on two days. After preliminary results showed no differences in allelic patterns due to tissue type or sex, entire animals minus brooded larvae were homogenized.

Agar overlays (10 ml of a 2% solution) were employed for all enzyme assays except AAT, G3PDH, and LAP, for which aqueous solutions were used. Standard recipes for all systems are in Shaw & Prasad (1970), Brewer (1970), and Poulik (1957).

Gels were scored as described in Ayala *et al.* (1973). First, independent loci were identified (Tracey *et al.,* 1975). The alleles of each locus were identified by the distance, in mm, that they migrated with respect to the most common allele of a reference population, which was given the arbitrary number 100. The time period for each run of a particular enzme was standardized (Hoagland & Turner, 1981). *Crepidula fornicata* from St. Mary's, Bridgeport, Connecticut, was the reference population because it was abundant, easily collected, and highly monomorphic. Reference samples of *C. convexa* and *C. plana* from Bridgeport, Connecticut, were also run with the corresponding questionable specimens from Florida, for direct comparison. Assignment of electrophoretic patterns to loci and consequent interpretations were made with the aid of data collected on the same enzyme systems for mollusks (Davis *et al.,* 1981) and other organisms (Lewontin, 1974). Heterozygote banding patterns corresponded with the subunit compositions of the enzymes.

Calculations were made of the allele frequencies at each locus. Rogers' (1972) and Nei's (1972) genetic distances were computed, and phenograms were constructed by computer, using unweighted arithmetic averaging. A minimum spanning tree was constructed using the NT-SYS computer package (Rohlf *et al.,* 1972) from both the Rogers' and Nei's distances. Other multivariate calculations were performed on the data, including multidimensional scaling (Sneath & Sokal, 1973). The percent of diagnostic loci (Ayala & Powell, 1972) was calculated as a means of determining distinctiveness of the populations from one another.

RESULTS

Biochemical Genetic Divergence among *Crepidula* Populations

The allele frequencies for all populations for which the sample size exceeded 50 are given in the Appendix. Table 3 lists, for each locus, the number of alleles unique to one taxon including *Crepidula aculeata* and *C. lingulata,* which had sample sizes of only 10 individuals each. The unique alleles are often but not always at low frequency. The estimates are conservative in that not all unique alleles are revealed by starch-gel electrophoresis (Coyne, 1976). The data are dependent upon the taxa selected. Increasing the sample size decreases the number of unique alleles, as rare alleles are found to be shared by the taxa.

Inspection of the Appendix reveals that the three Floridian populations purported to be *C. convexa* share the most common allele at all loci, but based on specimens electrophoresed in this study, share no alleles with the northern populations of *C. convexa* at 12 of the 24 loci examined, namely: AcPh, Adkin, EST NA II, GPI, HEX, LAP I and II, MPI I, MDH I and II, Pep G, and 6PGDH. The Ft. Pierce and northern populations of *C. plana* are not as distinct, but still share no alleles at 4 loci: AO, EST NA II, LAP I, and SOD. They differ in the most common allele at Pep T I, LDH, and MPI I.

TABLE 3. Number of alleles unique to one of 9 taxa. The taxa considered were *C. fornicata, C. onyx, C. protea, C. convexa, C.* cf. *convexa* of Florida, *C. plana, C.* cf. *plana* of Ft. Pierce, *C. lingulata,* and *C. aculeata.* The actual alleles for each taxon are listed in the Appendix, with the exception of *C. lingulata* and *C. aculeata.* Only 10 individuals of those species have been electrophoresed to date.

Locus	Total number of alleles	Unique alleles	Locus	Total number of alleles	Unique alleles
AcPh	10	9	MPI I	9	2
Adkin	7	5	MPI II	8	2
AO	5	2	MDH I	12	5
AAT	8	2	MDH II	11	6
EST NA I	9	5	Pep T I	10	3
EST NA II	9	5	Pep T II	8	3
G6PDH	3	2	Pep G	8	1
GPI	13	3	PGM	8	0
HEX	7	5	6PGDH	9	6
LDH	4	3	SoDH	6	4
LAP I	7	2	SOD	8	5
LAP II	8	4	XDH	4	1

TABLE 4. Comparisons of taxa based on allele differences. Above the diagonal: percent of 24 loci at which the 2 taxa share no alleles. Below the diagonal: percent of loci at which the most common allele differs for the 2 taxa, but some alleles are shared. On the diagonal: the same comparisons, but for populations within a species. Loci with 2 equally abundant alleles are omitted, as are loci with missing data.

	Proportion of loci with no allele in common						
	C.f.	*C.c.*	*C.* cf. *c.*	*C.p.*	*C.* cf. *p.*	*C.o.*	*C.pr.*
C. fornicata	.00 .00	.25	.42	.33	.37	.43	.54
C. convexa	.54	.00 .05	.50	.58	.54	.61	.63
C. cf. *convexa*	.37	.04	.00 .00	.63	.58	.78	.75
C. plana	.33	.33	.29	.04 .12	.17	.65	.50
C. cf. *plana*	.25	.33	.29	.17	.00 .04	.48	.63
C. onyx	.30	.26	.13	.04	.35	— —	.65
C. protea	.25	.21	.08	.33	.21	.13	— —
	Proportion of loci with different most common alleles, but some alleles shared.						

Table 4 allows one to make pairwise comparisons of this sort for all the taxa. The most common allele used in inter-species comparisons is determined by pooling the data for all populations of one species. In most but not all cases, the most common allele in each population is also the most common allele in the pooled sample. The most common alleles in the respective populations of each species were compared in order to calculate the values on the diagonal of Table 4. The percentages of loci which appear to have diverged completely (data above the diagonal) are no higher than 4% for populations of the

TABLE 5. Total percentage of divergent loci.

7 populations, *C. fornicata*	0%
3 populations, *C. convexa*	5
3 populations, *C.* cf. *convexa*	0
3 populations, *C. plana*	16
2 populations, *C.* cf. *plana*	4
C. convexa vs. *C.* cf. *convexa*	54
C. plana vs. *C.* cf. *plana*	34
C. cf. *plana* vs. *C. fornicata**	62
C. cf. *convexa* vs. *C. plana**	92

*Representing lowest and highest percentages, all interspecific comparisons.

same species, but are between 25% and 78% for interspecific comparisons. In comparison, the questionable taxa gave values of 50% for *C. convexa* vs. *C.* cf. *convexa* and 17% for *C. plana* vs. *C.* cf. *plana.*

Summing the mirror-image values above and below the diagonal gives the total percentage of divergent loci (Table 5). This calculation yields virtually the same result as the percent of diagnostic loci of Ayala & Powell (1972), which is shown in Table 6. The boldface values in Table 6 are those that compare *C. convexa* and *C.* cf. *convexa* and those that compare *C. plana* and *C.* cf. *plana.* The percent diagnostic loci within a species is no higher than 4.3% (representing a single locus). Interspecific comparisons, on the other hand, vary from 58.3 to 91.3% at the 99% certainty level. Comparison of *C. convexa* and *C.* cf. *convexa* yields values of 54.2 to 60.9%, while that of *C. plana* and *C.* cf. *plana* yields 25.0–29.2% diagnostic loci. Use of percent diagnostic loci is slightly more conservative than the percentage of divergent loci and is a less ambiguous method. Both *C.* cf. *convexa* and *C.* cf. *plana* diverge from the northern populations that had been thought to be the same species by more than would be expected if they were conspecific, but by less than the known congeneric pairs. In fact, the *C. plana-C.* cf. *plana* value is midway between the percent diagnostic loci for intra- and inter-specific comparisons. The *C.convexa* comparison is much closer to the values for different species in the genus as surveyed in this report.

Of the 24 loci of *Crepidula* cf. *convexa,* 50% share no common alleles with *C. convexa* but 44% have the same major alleles at about the same frequencies (Appendix); only one locus has a different major allele but some shared alleles. This pattern implies stasis at half the loci but major evolutionary change at the others. The assemblage of loci showing divergence between *C.* cf. *convexa* and *C. convexa* is not the same as that showing divergence between *C. plana* and *C.* cf. *plana* or between other taxa

The allele frequencies were used to calculate the Nei's genetic distances and Rogers' distances given in Table 7. The phenogram resulting from Rogers' distance using unweighted arithmetic averaging is shown in Figure 1; its cophenetic correlation coefficient was 0.988. A similar phenogram resulted from Nei's distance, except that the 1980 and 1981 samples of *Crepidula fornicata* taken at Bridgeport, Connecticut, were adjacent. The correlation was 0.927.

The populations of *C. fornicata* cluster together at Rogers' distance values of ≤ 0.076. The year-to-year change in gene frequencies (coupled with sampling error) of 0.052 is as great as several of the between-population distances in this group of New England populations with planktonic larvae (Table 7). The three populations of *C. plana* form a separate cluster, as do the two of *C.* cf. *plana,* the three of *C. convexa,* and the three of *C.* cf. *convexa,* all at averaged values of 0.100 to 0.141 (Fig. 1). The first two clusters merge at 0.354; the last two merge at 0.531. Hence *C.* cf. *plana* is allozymically more closely related to *C. plana* than is *C.* cf. *convexa* to *C. convexa.* In both *C. plana-C.* cf. *plana* and *C. convexa-C.* cf. *convexa,* the pairs are more closely related to each other than to any of the other taxa examined. Something of the genetic variation within *C.* cf. *convexa* can be seen by comparing the three electrophoresed populations. The Rogers' distance between adjacent populations at Ft. Pierce is 0.041; the distances between those and the Gulf Breeze population in another body of water are 0.068 and 0.076. *Crepidula fornicata* has the greatest genetic similarity to *C. plana*; it is most distantly related to *C. convexa.* The other species, *C. onyx* and *C. protea,* are genetic as well as geographic outlyers, not forming any clusters, although they coalesce with *C. fornicata* and *C. plana* slightly ahead of *C. convexa.*

A minimum spanning tree with subsets was constructed using the Rogers' distance data (Fig. 2). The minimum spanning tree gives the maximum diameters of clusters and minimum gaps between clusters. The maximum diameter of the cluster of *C. fornicata* is 0.076, that of *C. plana* proper, 0.146, and that of *C. plana*

TABLE 6. Percent diagnostic loci. Above the diagonal: 99.9% probability. Below the diagonal: 99% probability.

	Crepidula fornicata							*Crepidula convexa*			*C.* cf. *convexa*			*Crepidula plana*			*C.* cf. *plana*		*C. onyx*	*C. protea*
	Br. 1980	Br. 1981	M.B. L.B.	W.H. Y.C.	Mill-stone	L.C.	K.C.	Br.	Mill-stone	L.C.	L.J.C.	B.H.	G.B.	Br.	Mill-stone	L.C.	L.J.C.	B.H.	Balboa	Rio Grande Brasil
Crepidula fornicata																				
Br. 1980		0	0	0	0	0	0	58.3	70.8	69.6	79.2	79.2	75.0	54.2	45.8	58.3	58.3	54.2	73.9	60.9
Br. 1981	0		0	0	0	0	0	58.3	70.8	69.6	70.8	70.8	62.5	58.3	45.8	54.2	54.2	54.2	60.9	60.9
M.B.L.B.	0	0		0	0	0	0	54.2	66.7	60.9	70.8	66.7	66.7	54.2	45.8	54.2	58.3	54.2	69.6	60.9
W.H.Y.C.	0	0	0		0	0	0	54.2	66.7	65.2	75.0	75.0	66.7	58.3	45.8	54.2	58.3	54.2	65.2	65.2
Millstone	0	0	0	0		0	0	54.2	66.7	65.2	70.8	70.8	62.5	58.3	45.8	62.5	58.3	58.3	65.2	65.2
L.C.	0	0	0	0	0		0	58.3	70.8	65.2	79.2	75.0	70.8	58.3	45.8	66.7	62.5	58.3	69.6	69.6
K.C.	0	0	0	0	0	0		50.0	62.5	56.5	70.8	66.7	58.3	54.2	45.8	58.3	58.3	54.2	60.9	69.6
Crepidula convexa																				
Br.	66.7	66.7	62.5	62.5	66.7	66.7	66.7		0	0	**54.2**	**54.2**	**54.2**	70.8	62.5	70.8	70.8	62.5	65.2	78.3
Millstone	75.0	75.0	70.8	70.8	70.8	75.0	75.0	0		4.3	**54.2**	**54.2**	**54.2**	79.2	70.8	75.0	75.0	70.8	69.6	78.3
L.C.	78.3	82.6	73.9	73.9	73.9	78.3	78.3	0	4.3		**56.5**	**56.5**	**56.5**	87.0	78.3	73.9	73.9	69.6	77.3	77.3
Crepidula cf. *convexa*																				
L.J.C.	79.2	79.2	79.2	79.2	75.0	79.2	79.2	**54.2**	**54.2**	**56.5**		0	0	87.5	75.0	87.5	75.0	70.8	82.6	78.3
B.H.	79.2	79.2	79.2	79.2	75.0	79.2	79.2	**54.2**	**54.2**	**56.5**	0		0	87.5	75.0	83.3	75.0	66.7	78.3	78.3
G.B.	75.0	75.0	79.2	75.0	70.8	79.2	75.0	**58.3**	**58.3**	**60.9**	0	0		83.3	75.0	83.3	75.0	70.8	78.3	73.9
Crepidula plana																				
Br.	62.5	58.3	62.5	58.3	66.7	66.7	62.5	79.2	79.2	91.3	87.5	87.5	83.3		4.2	4.2	**25.0**	**20.8**	73.9	65.2
Millstone	58.3	58.3	58.3	58.3	58.3	62.5	58.3	79.2	83.3	91.3	87.5	87.5	83.3	4.2		0	**25.0**	**20.8**	69.6	65.2
L.C.	66.7	62.5	62.5	62.5	66.7	66.7	62.5	75.0	75.0	82.6	87.5	87.5	83.3	4.2	0		**29.2**	**29.2**	78.3	65.2
Crepidula cf. *plana*																				
L.J.C.	62.5	62.5	62.5	62.5	62.5	62.5	62.5	75.0	79.2	82.6	83.3	83.3	79.2	**25.0**	**29.2**	**29.2**		0	65.2	73.9
B.H.	58.3	58.3	58.3	58.3	62.5	58.3	58.3	75.0	79.2	82.6	75.0	75.0	75.0	**25.0**	**29.2**	**29.2**	0		60.9	73.9
Crepidula onyx																				
Balboa	73.9	69.6	69.6	69.6	69.6	69.6	69.6	69.6	73.9	81.8	82.6	82.6	78.3	82.6	82.6	82.6	73.9	78.3		63.6
Crepidula protea																				
Brasil	69.6	69.6	69.6	69.6	69.6	69.6	69.6	82.6	78.3	81.8	82.6	82.6	82.6	69.6	73.9	69.6	82.6	73.9	68.2	

C. convexa (Little Compton), *C. onyx*, and *C. protea* each lack a different locus. Otherwise, there are 24 loci per population.

TABLE 7. Genetic distances. Nei's distances are above the diagonal; Rogers' distances are below the diagonal. Abbreviations of localities are explained in Table 1.

	Crepidula fornicata							*Crepidula convexa*			*Crepidula* cf. *convexa*			*Crepidula plana*			*C.* cf. *plana*		*C. onyx*	*C. protea*
	Br 80	Br 81	MBL	WH	M	LC	K	Br	M	LC	LJC	BH	GB	Br	M	LC	LJC	BH	B	RG
C.f. Br 80	*	0.006	0.007	0.016	0.012	0.006	0.009	1.282	1.510	1.484	1.461	1.457	1.281	1.124	1.024	1.110	1.055	1.145	1.329	1.486
C.f. Br 81	0.052	*	0.004	0.010	0.008	0.004	0.007	1.256	1.481	1.450	1.431	1.427	1.243	1.076	0.976	1.063	1.007	1.092	1.333	1.466
C.f. MBL	0.050	0.048	*	0.008	0.006	0.003	0.006	1.225	1.458	1.418	1.419	1.414	1.249	1.067	0.969	1.056	1.006	1.097	1.380	1.482
C.f. WH	0.076	0.073	0.054	*	0.011	0.011	0.014	1.225	1.463	1.433	1.420	1.416	1.242	1.113	1.008	1.080	1.045	1.106	1.321	1.503
C.f. M	0.064	0.056	0.047	0.066	*	0.009	0.016	1.279	1.495	1.474	1.394	1.389	1.216	1.109	0.999	1.072	1.029	1.097	1.356	1.461
C.f. LC	0.048	0.051	0.034	0.056	0.058	*	0.005	1.273	1.499	1.466	1.467	1.462	1.287	1.109	1.007	1.097	1.041	1.128	1.349	1.486
C.f. K	0.067	0.061	0.053	0.062	0.081	0.049	*	1.213	1.450	1.415	1.445	1.439	1.263	1.069	0.967	1.062	1.009	1.098	1.327	1.533
C.c. Br	0.712	0.699	0.698	0.699	0.707	0.711	0.697	*	0.037	0.052	0.723	0.725	0.786	2.025	1.962	2.232	1.693	1.916	1.787	1.704
C.c. M	0.747	0.734	0.739	0.732	0.738	0.750	0.741	0.100	*	0.057	0.743	0.746	0.800	2.346	2.254	2.526	1.855	2.068	1.921	1.683
C.c. LC	0.749	0.735	0.734	0.736	0.742	0.748	0.734	0.116	0.106	*	0.764	0.766	0.821	2.254	2.158	2.502	1.800	2.116	1.975	1.710
C. cf. *c.* LJC	0.750	0.741	0.743	0.737	0.734	0.753	0.748	0.515	0.522	0.531	*	0.008	0.068	2.241	2.004	2.239	1.879	1.782	2.207	1.854
C. cf. *c.* BH	0.743	0.733	0.736	0.729	0.726	0.746	0.741	0.515	0.522	0.528	0.041	*	0.076	2.241	2.043	2.182	1.839	1.752	2.182	1.891
C. cf. *c.* GB	0.703	0.691	0.696	0.687	0.684	0.704	0.700	0.546	0.546	0.558	0.110	0.123	*	2.172	1.905	2.199	1.847	1.718	2.142	1.736
C.p. Br	0.668	0.646	0.648	0.661	0.655	0.662	0.648	0.827	0.856	0.849	0.853	0.845	0.840	*	0.097	0.096	0.334	0.366	2.036	1.580
C.p. M	0.644	0.622	0.624	0.638	0.630	0.637	0.623	0.831	0.860	0.852	0.844	0.843	0.826	0.146	*	0.052	0.401	0.426	2.001	1.600
C.p. LC	0.656	0.635	0.638	0.637	0.631	0.652	0.645	0.855	0.865	0.876	0.859	0.845	0.849	0.135	0.108	*	0.408	0.424	2.056	1.497
C. cf. *p.* LJC	0.645	0.625	0.626	0.637	0.629	0.637	0.627	0.785	0.807	0.804	0.822	0.808	0.812	0.310	0.357	0.355	*	0.045	1.680	1.710
C. cf. *p.* BH	0.667	0.648	0.652	0.645	0.642	0.663	0.654	0.810	0.818	0.829	0.795	0.780	0.782	0.344	0.390	0.367	0.118	*	1.677	1.666
C. o. B	0.698	0.694	0.710	0.701	0.698	0.711	0.707	0.789	0.802	0.811	0.844	0.834	0.829	0.817	0.821	0.821	0.781	0.760	*	1.397
C. pr. RG	0.758	0.746	0.756	0.756	0.742	0.761	0.765	0.788	0.784	0.793	0.829	0.824	0.792	0.783	0.792	0.761	0.796	0.778	0.721	*

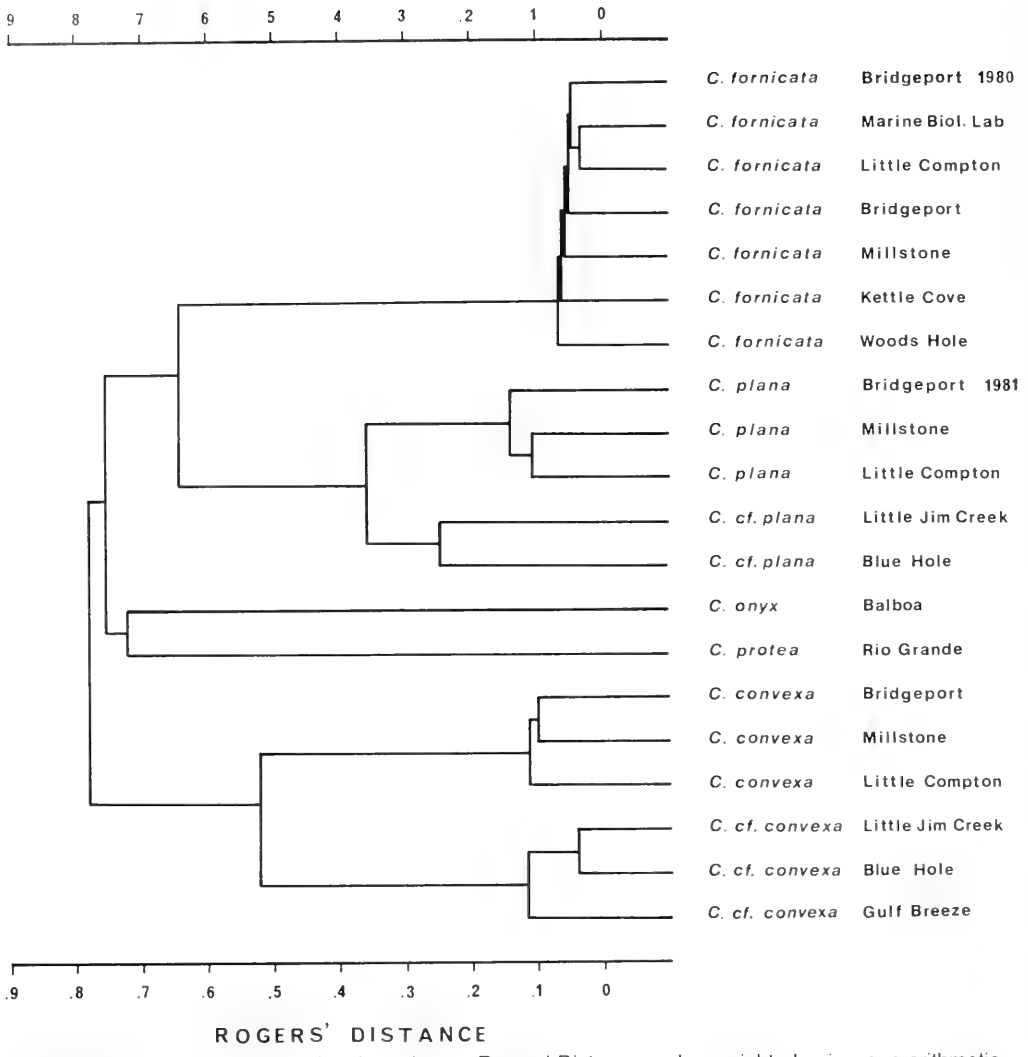

FIG. 1. Phenogram of *Crepidula* data based upon Rogers' Distance and unweighted pair-group arithmetic averaging.

plus *C.* cf. *plana,* 0.390. The maximum diameter for *C. convexa* proper is 0.116 while that of all 6 samples referred to *C. convexa* is 0.558.

Additional manipulation of the data using the NT-SYS computer package produced a phenogram based on multidimensional scaling. It was virtually the same as the phenogram based directly on Rogers' distance. The correlation coefficient of a minimum spanning tree on multidimensional scaling was 0.946. When the same technique was used on Nei's distance, which is not a metric, a very different phenogram based on multidimensional scaling was obtained. Its correlation coefficient with a minimum spanning tree was only 0.850, and there were obvious distortions in the phenogram, such as the placement of populations of the same species in different clusters.

Nei's distance (D) values (Table 7) are important for comparison with other studies, despite their poorer performance in clustering the data when compared with the less-frequently-used Rogers' distance. The populations of *C. fornicata* had D values among themselves of 0.003 to 0.016, averaging 0.008. The three Floridian populations of *C.*

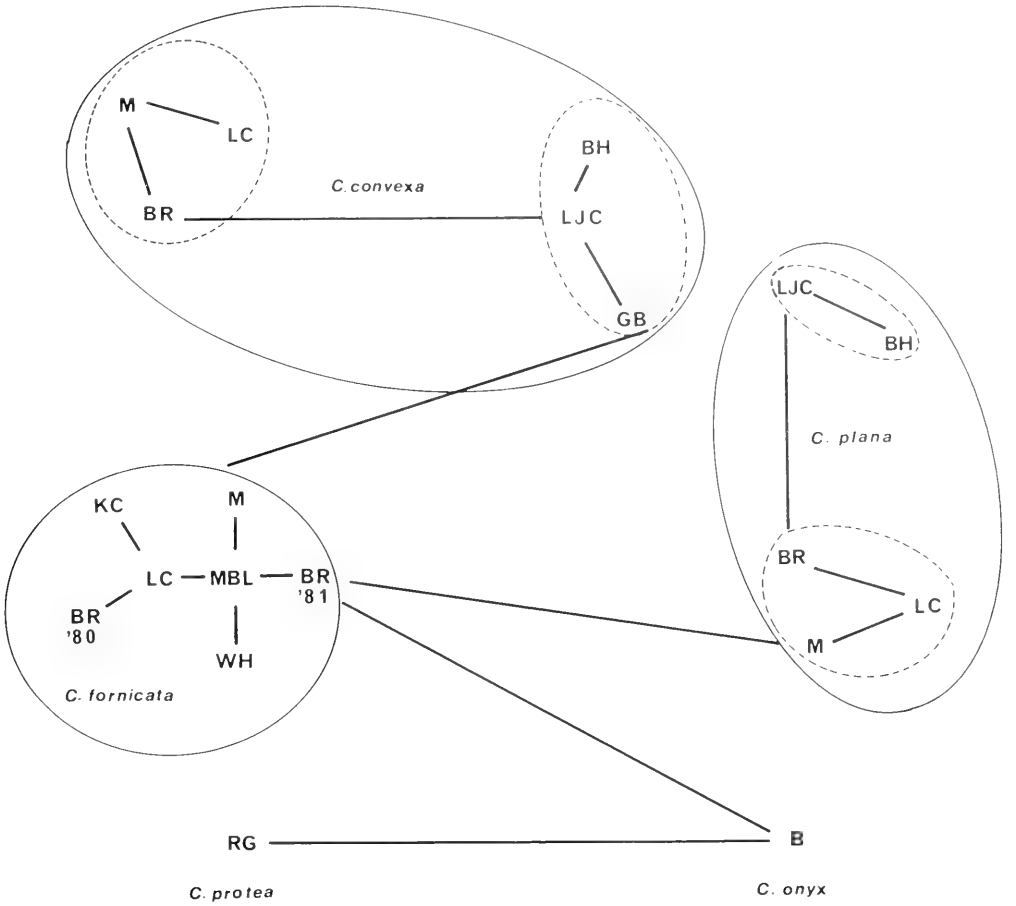

FIG. 2. Minimum spanning tree diagram based upon Rogers' Distance, with subsets superimposed. Lines are drawn to scale, showing relative distances between taxa.

cf. *convexa* were separated by D = 0.008–0.076; the separation of the 3 populations of northern *C. convexa* was 0.037–0.057. The two groups coalesced at 0.764. For southern and northern *C. plana,* D values were 0.045 and 0.052–0.097, respectively; the groups coalesced at 0.393.

Ecology and Life History

Crepidula plana was collected on *Strombus gigas* on mud-sand flats in Biscayne Bay, Florida. The specimens brooded young of the type released as planktonic veligers, as is characteristic of *C. plana* from New England. Although the specimens died before they could be frozen for electrophoresis, this find indicates that true *C. plana* exists in the southern United States. *C. plana* was quite rare at the locality sampled; collecting over a period of 3 hours by 6 persons yielded only 10 specimens.

Specimens of both *Crepidula* cf. *convexa* and *C.* cf. *plana* were found in the shallow waters of the Indian River between mangrove islands and in muddy and grassy areas adjacent to oyster reefs. *C.* cf. *plana* was most commonly on the insides of empty beer cans, but was also on the outside of cans and in the aperture of dead shells, including *Strombus gigas.* Such a habitat is indistinguishable from that of true *C. plana* in Biscayne Bay, Florida, as well as in New England. *Crepidula* cf. *convexa* was found most commonly on the exterior of living *Cerithium atratum* Born occupying grassy areas below the oyster

reefs. Specimens were also abundant and quite large on the outsides of beer cans, *Strombus,* and waterlogged mangrove wood. This range of substrate is typical of true *C. convexa.* The major difference in habitat between the two Ft. Pierce species is that *C.* cf. *convexa* is more often found on living and mobile substrates. *C.* cf. *convexa* from Gulf Breeze, Florida, apparently came into the laboratory's saltwater system to settle in oyster tanks. I have not observed it there in its natural habitat. Gulf Breeze specimens grew on unrestricted substrates and were larger than those from Ft. Pierce.

Crepidula cf. *plana* from Ft. Pierce could not be distinguished from the New England *C. plana* by shell characters or by superficial anatomical characters. However, mature specimens tended to be smaller, even when occupying unrestricted substrates. The larvae being brooded by *C. plana* from Ft. Pierce were of the developmental type that hatch not as veligers, but as metamorphosed young. Of 15 broods examined, there were between 12 and 30 egg sacs per brood, averaging 22. There was an average of 7 large, yolky eggs or developing embryos per sac, for an average brood size of about 150. The exact egg size cannot be given until uncleaved eggs are observed, but it is similar to that of *C. convexa.* The number of embryos per capsule appeared to decline with developmental stage. By comparison, true *C. plana* produces about 8,000 veligers per brood.

The larval development of *C.* cf. *convexa* from Florida also differed from that of its northern relative. Laboratory observations revealed that, instead of releasing metamorphosed young, the larvae of both east and west Florida populations were released in a partially metamorphosed condition akin to the pediveliger state of some bivalves. No other *Crepidula* has been reported to release pediveligers. The young came out of the brood sacs crawling, but still with a small bilobed velum. The velum was held at right angles to the foot in front of the animal, which resembled a propeller-driven airplane taxiing for take-off. The velum was used to stabilize the animal and in conjunction with the foot, to right it if it turned over. It might also be used for feeding. Swimming in slow circles was accomplished only rarely and briefly. When contracted under the margin of the shell, the velum appeared as two yellowish dots behind and lateral to the eyes. The color is due to dense pigment granules that outline the velum.

Twenty brooding females of *Crepidula* cf. *convexa* from Ft. Pierce had an average of 14.5 (± S.D. of 5.0) egg sacs (range: 6–27). There were 1–16 embryos per sac, averaging 7.2 ± 1.9. Brood size is thus about 100. By direct count, the smallest brood was 60 embryos; the largest was 224. Brood size for the larger Gulf Breeze specimens averaged 14.9 ± 4.5 egg sacs (range: 7–26) and 9.9 ± 2.2 embryos per sac for a mean total of 148 per brood. The average brood size for New England *C. convexa* of two years old is about 200 (Hoagland, 1975). The embryos of *C.* cf. *convexa* were 0.63–0.70 mm in diameter at hatching, whereas *C. convexa*'s are about 1 mm. As in *C.* cf. *plana,* brood size declines with age of the embryos. No uncleaved eggs were available to measure egg size of Floridian specimens.

DISCUSSION

The type-locality of *Crepidula convexa* Say is "coast of the United States," and the type is lost. The name *C. convexa* is well-established in the literature for the specimens from New England and the middle-Atlantic states. If a new species is erected the Floridian taxon should receive the new name. Similarly, the type-locality of *C. plana* Say is "coast of the United States." Say (1882) stated that it was found in Maryland, Carolina, Georgia, E. Florida, and the shores of New Jersey. Because only the taxon with planktonic larvae is known from New Jersey and Maryland, it should retain the name *C. plana.*

Electrophoresis has been used successfully to distinguish sibling species (e.g. Manwell & Baker, 1963; Grassle & Grassle, 1976; Murphy, 1978; Halliday, 1981; Bucklin & Hedgecock, 1982). Often, as in this present study, the first suggestion that sibling species may exist has come from an observed difference in reproduction (Mastro *et al.,* 1982). In most cases, the cryptic species has been sympatric with the named species, hence biochemical data showing genetic isolation are sufficient for one to conclude the existence of two species. That is not the case for *C.* cf. *plana* and *C.* cf. *convexa.* It is difficult to draw conclusions based upon the biological species concept when populations are allopatric, regardless of whether electrophoretic data or traditional morphological data are used. However, one can make a reasonable guess as to the distinctiveness of the gene

pools of two allopatric taxa by using molecular genetics to identify distinct lineages.

It is instructive to compare Nei's distance (D) values obtained for *Crepidula* with those obtained for other taxa. Published genetic distances between sibling species pairs reviewed by Ryman *et al.* (1979) include 0.759 for the freshwater snail *Goniobasis* (Chambers, 1978), 0.466 to 0.788 for *Drosophila* (Ayala, 1975), 0.232 for *Dicamptodon* species (Daugherty & Allendorf, 1977), and only 0.025 for *Salmo trutta* (Ryman *et al.*, 1979). Templeton (1980) cites several references in which speciation occurred with very little divergence at enzyme-coding loci. However, Coyne (1976) cautioned that use of alternative electrophoretic techniques can reveal previously hidden genetic divergence.

Davis (1983) summarized data for congeneric species of Unionidae and Sphaeriidae (freshwater bivalves), obtaining D values ranging from 0.01 to 1.06, while D values for 29 populations within several species ranged from 0.001 to only 0.184. He concluded that for Unionidae and Sphaeriidae, the probability is good that two taxa are different species if $D \geq 0.222$. Ayala (1975) in reviewing other data, concluded that genetic identity values ≥ 0.97 ($D \leq 0.031$) are common for populations of a species, but those lower than 0.90 ($D \geq 0.11$) are rare.

By these guidelines together with analysis of D values for known species and populations of *Crepidula,* both *Crepidula* cf. *convexa,* whose populations had distances of 0.723 to 0.821 from populations of *C. convexa,* and *C.* cf. *plana,* whose populations had D values of 0.334 to 0.426 from *C. plana,* are genetically distinct enough to be candidates for species status. Certainly, gene exchange does not now exist between the respective taxa. One can conclude also that the populations of *C. fornicata* (D between populations ≤ 0.016), *C. convexa* ($D \leq 0.057$) and *C. plana* ($D \leq 0.097$) represent three distinct gene pools (Table 7; Fig. 1).

Although some authors have shown speciation to occur without much genetic divergence, evidence that large-scale genetic divergence on the order of $D > 0.30$ can occur in *Crepidula* without speciation is lacking. It is just possible that *Crepidula plana* and *C.* cf. *plana* have not diverged in characters essential to reproductive compatibility. However, the coupling of the genetic distance and percent divergent loci with the information on larval differences makes this possibility exceedingly unlikely. The production of viable offspring from parents with vastly different larval developmental patterns would be required.

It is even more unlikely that *C. convexa* and *C.* cf. *convexa* are one species under the biological species concept of the ability to produce viable offspring, when fully half of the randomly-chosen enzyme loci have diverged and major larval differences are also seen. In no other species of *Crepidula* have I observed a comparable stage of development that includes well-developed shell and foot with velum still present. In all the hundreds of broods of *C. convexa* from New England to New Jersey that I have examined (Hoagland, 1975), none of the females released pediveligers. Hence, there is a major difference in developmental pattern between the Floridian and northern taxa.

The genetic distance between the Gulf Breeze and the Fort Pierce populations of *Crepidula* cf. *convexa* is within a reasonable range for a single species. The much closer genetic relationship of the two samples within the mangrove system at Ft. Pierce is also within expectations. The genetic and reproductive similarities of the Gulf Breeze and Ft. Pierce populations, so distinct as to be in separate bodies of water, make all but inevitable the conclusion that these populations form a taxon deserving of species status.

The Gulf Breeze sample is more distant genetically and geographically from true *Crepidula convexa* than are the Ft. Pierce samples. In fact, the more geographically distant the populations of the *C. convexa* complex are from one another, the greater the genetic distance between them (Table 7). Similarly, *C.* cf. *plana* from Florida is most similar genetically to the population sample of *C. plana* that is closest geographically, among those electrophoresed.

The finding that genetic distances between known species of *Crepidula* are greater than those for the two pairs of taxa under study, coupled with the shell-shape and ecological similarities within the pairs, could indicate that the two Floridian taxa and their respective New England counterparts diverged relatively recently. However, this hypothesis cannot be tested directly because the species pairs cannot be identified in the fossil record. *Crepidula plana* and *C.* cf. *plana* are more disparate in larval development than the other pair of taxa, yet show about half as much genetic divergence. It is not possible to say whether the

change in larval development was primary, or a secondary result of the isolation of the southern and northern populations.

It may be that the gene pool of *Crepidula convexa* began to fragment earlier than that of *C. plana.* Alternatively, *C. convexa* may have diverged to a greater extent due to greater ecological differentiation or the greater chance for isolation of small populations of a species without a long-term planktotrophic larval stage. The large number of loci in *C.* cf. *convexa* that are either fixed for an alternative allele or that do not share any of several alleles with *C. convexa* may indicate strong forces of either selection or founder/bottleneck effects. The almost equal number of unchanged loci, many with several alleles at more or less the same frequencies, perhaps indicates stabilizing selection. This U-shaped pattern of allele variation at a number of loci is commonly found when comparing two closely-related species (Ayala, 1975). Some argue that such a pattern is compatible with neutral models of allozyme patterns (Chakraborty *et al.,* 1978).

Geographical isolation of *Crepidula convexa* and *C.* cf. *plana* is easily envisioned from the lack of planktonic larval dispersal and the more or less sedentary nature of adults. Patchy substrate is also a factor potentially isolating populations of *Crepidula.* However, adults of all species can be transported on moving substrates.

The data on allele frequencies within one population collected over two years, and between adjacent localities such as Blue Hole and Little Jim Creek in the same time period, show that some local variation in time and space do occur regardless of species. The year-to-year variation in *Crepidula fornicata* from Bridgeport is on the same scale as that between populations in New England, leading to the general conclusion that the New England populations are not genetically distinguishable. However, analysis of individual allele frequencies could still show patterns that would contradict this finding. The genetic uniformity and relatively low polymorphism of *C. fornicata* is in contrast to the population genetic structure of both the *C. plana* and *C. convexa* groups that has allowed isolated gene pools to develop. It is tempting to say that the genetic closeness of populations of *C. fornicata* is due to their planktonic larval development, compared with *C. convexa,* for example, which has greater genetic distances between populations. However, on further inspection, *C. plana* breaks the pattern: it has planktonic larvae, but genetic distances between populations greater than those of *C. convexa.*

Some of the variation between adjacent populations in time and space is due to the presence of rare alleles. The same allele is often more common in another population or species, although some of the rare alleles are unique to one geographical region. The detection of rare alleles depends upon a large sample size, although Gorman & Renzi (1979) have shown that reliable genetic distances can be obtained for as few as 10 individuals per taxon, because genetic distance is little affected by rare alleles.

The genetic relationships based on Rogers' distance and a simple phenogram (Fig. 1) were nearly identical with those determined by Nei's distance (Table 7). Advantages of the latter are its comparability with other studies, its known statistical properties, and its theoretical appeal. Rogers' distance imparts less distortion to further analyses of this particular data set, but other authors have found Nei's distance superior (Davis, 1984).

The analysis of the number of loci which have divergent allele frequencies (Tables 4 and 5) and the present diagnostic loci (Table 6) serve to delineate taxa. In the process of tabulating the data, I noted which enzyme loci have diverged. For, example, PGM has 8 alleles for the 9 taxa (including *Crepidula lingulata* and *C. aculeata*) examined; none is unique to one taxon. But of 10 AcPh alleles, 9 are unique to one taxon (Table 3). Molecular divergence does not occur at the same rate at all loci for a given set of taxa, as has been discussed by Avise (1976), Gillespie & Langley (1974) and others. Furthermore, the set of loci that has diverged between *C. convexa* and *C.* cf. *convexa* differs considerably from the set that has diverged between *C. plana* and *C.* cf. *plana.* From the lists of these divergent enzyme loci on p. 611, one can see that only EST NA II and LAP I show complete divergence in both sets of taxa. Pep G and MPI I share no alleles between *C. convexa* and *C.* cf. *convexa* but share a few minor alleles between *C. plana* and *C.* cf. *plana.* Twelve other loci are divergent in one but not the other pair of taxa. This finding implies that microevolution of allozymes can proceed quite differently in pairs of congeners living today in habitats with similar general ecological requirements.

Anatomical data are required for the formal

description of new taxa resulting from this work. Of course, anatomical traits, like allele frequencies, vary to some extent among potentially interbreeding populations that compose a species.

Since breeding compatibility is not known for allopatric populations, three methods can be employed to apply the biological species test to these taxa. First, areas in Florida (particularly Biscayne Bay), Virginia and North Carolina can be searched in the hope of finding sympatric populations to be electrophoresed. Second, specimens from Florida can be reared with those from northern populations to see if offspring are produced. Third and more simply, healthy snails of the two genetic types can be maintained together to see if opposites form male-female pairs due to species-specific pheromonal attraction. If not, there is evidence that effective barriers to reproduction exist in these copulating snails.

Conclusions and Future Work

On the basis of electrophoresis and life history analysis, two allopatric species pairs have been tentatively identified. It appears that a prediction that more than one species of white, flat *Crepidula* exists in the Western Atlantic has been verified. Formal species descriptions await anatomical studies in progress. A species complex including several more species may be found as populations supposed to be *Crepidula convexa* are investigated in Texas and the Caribbean islands. The technique of electrophoresis is also being used for an in-depth study of the white, flat species of *Crepidula*.

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LITERATURE CITED

AVISE, J. C., 1976, Genetic differentiation during speciation. *In* AYALA, F. J., ed., *Molecular Evolution,* p. 106–122. Sinauer Associates, Sunderland, Massachusetts.

AYALA, F. J., 1975, Genetic differentiation during the speciation process. *In* DOBZHANSKY, T., HECHT, M. K. & STEER, W. E., eds., *Evolutionary Biology,* 8: 1–78. Plenum Press, New York.

AYALA, F. J. & POWELL, J. R., 1972, Allozymes as diagnostic characters of sibling species of *Drosophila. Proceedings of the National Academy of Sciences, U.S.A.,* 69: 1094–1096.

AYALA, F. J., HEDGECOCK, D., ZUMWALT, G. S. & VALENTINE, J. W., 1973, Genetic variation in *Tridacna maxima,* an ecological analog of some unsuccessful evolutionary lineages. *Evolution,* 27: 177–191.

BERRY, S. S., 1950, A partial review of some West American species of *Crepidula. Leaflets in Malacology,* Redlands, California, 1: 35–40.

BREWER, G. J., 1970, *An introduction to isozyme techniques.* Academic Press, New York, 186 p.

BUCKLIN, A. & HEDGECOCK, D., 1982, Biochemical genetic evidence for a third species of *Metridium* (Coelenterata, Actiniaria) *Marine Biology,* 66: 1–8.

CALABRESE, A. & RHODES, E. W., 1974, Culture of *Mulinia lateralis* and *Crepidula fornicata* embryos and larvae for studies of pollution effects. *Thalassia Jugoslavica,* 10: 89–102.

CHAKRABORTY, R., FUERST, P. A. & NEI, M., 1978, Statistical studies on protein polymorphism in natural populations. II. Gene differentiation between populations. *Genetics,* 88: 367–390.

CHAMBERS, S. M., 1978, An electrophoretically-detected sibling species of "*Goniobasis floridensis*" (Mesogastropoda: Pleuroceridae). *Malacologia,* 17: 157–162.

COE, W. R., 1936, Sexual phases in *Crepidula. Journal of Experimental Zoology,* 72: 455–477.

CONKLIN, E. G., 1897, The embryology of *Crepidula. Journal of Morphology,* 13: 1–226.

COYNE, J. A., 1976, Lack of genic similarity between two sibling species as revealed by various techniques. *Genetics,* 84: 593–607.

DAUGHERTY, C. H. & ALLENDORF, F. W., 1977, The taxonomic value of genetic distance. Data from two amphibians. *American Zoologist,* 17: 973.

DAVIS, G. M., 1983, Relative roles of molecular genetics, anatomy, morphometrics and ecology in assessing relationships among North American Unionidae (Bivalvia). *In* OXFORD, G. S. & ROLLINSON, D., ed., *Systematics Association Special Volume* 24, p.193–222. Academic Press, London.

DAVIS, G. M., 1984, Genetic relationships among some North American Unionidae (Bivalvia): sibling species, convergence, cladistic relationships. *Malacologia,* 25: 629–648.

DAVIS, G. M., HEARD, W., FULLER, S. L. H. & HESTERMAN, C., 1981, Molecular genetics and speciation in *Elliptio,* and its relationship to other taxa of North American Unionidae (Bivalvia). *Biological Journal of the Linnean Society,* 15: 131–150.

DILLON, R. T. & DAVIS, G. M., 1980, The *Goniobasis* of southern Virginia and northwestern North Carolina: genetic and shell morphometric relationships. *Malacologia,* 20: 83–98.

GALLARDO, C. S., 1977, Two modes of development in the morphospecies *Crepidula dilatata* (Gastropoda: Calyptraeidae) from southern Chile. *Marine Biology,* 39: 241–251.

GALLARDO, C. S., 1979, Especies gamelas del género *Crepidula* en la costa de Chile; una redescripción de *C. dilatata* Lamarck y descripción de *C. fecunda* n. sp. *Studies on Neotropical Fauna and Environment,* 14: 216–227.

GILLESPIE, J. H. & LANGLEY, C. H., 1974, A general model to account for enzyme variation in natural populations. *Genetics,* 76: 837–848.

GORMAN, G. C. & RENZI, J., Jr., 1979, Genetic distance and heterozygosity estimates in electrophoretic studies of sample size. *Copeia,* 1979: 242–249.

GRASSLE, J. P. & GRASSLE, J. F., 1976, Sibling species in the marine pollution indicator *Capitella* (Polychaeta). *Science,* 192: 567–569.

HALLIDAY, R. B., 1981, Heterozygosity and genetic distance in sibling species of meat ants (*Iridomyrmex purpureus* group). *Evolution,* 35: 234–242.

HOAGLAND, K. E., 1975, *Reproductive strategies and evolution in the genus* Crepidula. Ph.D. thesis, Harvard University, Cambridge, Mass., 350 p.

HOAGLAND, K. E., 1976, Patterns of evolution and niche partitioning in North American *Crepidula* (Gastropoda: Calyptraeidae). *American Malacological Union Bulletin for 1975:* 52–53.

HOAGLAND, K. E., 1977, Systematic review of fossil and recent *Crepidula* and discussion of evolution of the Calyptraeidae. *Malacologia,* 16: 353–420.

HOAGLAND, K. E., 1978, Protandry and the evolution of environmentally mediated sex change: a study of the Mollusca. *Malacologia,* 17: 365–391.

HOAGLAND, K. E., 1983, Ecology and larval development of *Crepidula protea* (Prosobranchia: Crepidulidae) from southern Brasil: A new type of egg capsule for the genus. *Nautilus,* 97: 105–109.

HOAGLAND, K. E. & TURNER, R. D., 1981, Evolution and adaptive radiation of wood-boring bivalves (Pholadacea). *Malacologia,* 21: 111–148.

KRÜGER, F., 1970, Untersuchungen über die Temperaturabhängigkeit des Sauerstoffverbrauchs von *Crepidula fornicata* (Mollusca: Prosobranchia). *Marine Biology,* 5: 145–153.

LEWONTIN, R. C., 1974, *The genetic basis of evolutionary change.* Columbia, New York, 346 p.

MANWELL, C. & BAKER, C. M. A., 1963, A sibling species of sea cucumber discovered by starch gel electrophoresis. *Comparative Biochemistry and Physiology,* 10: 39–53.

MASTRO, E., CHOW, V. & HEDGECOCK, D., 1982, *Littorina scutulata* and *Littorina plena*: sibling species status of two prosobranch gastropod species confirmed by electrophoresis. *Veliger,* 24: 239–247.

MURPHY, P. G., 1978, *Collisella austrodigitalis* sp. nov.: a sibling species of limpet (Acmaeidae) discovered by electrophoresis. *Biological Bulletin,* 155: 193–206.

NEI, M., 1972, Genetic distance between populations. *American Naturalist,* 106: 283–292.

POULIK, M. D., 1957, Starch gel electrophoresis in a discontinuous system of buffers. *Nature,* 180: 1477–1479.

ROGERS, J. S., 1972, Measures of genetic similarity and genetic distance. *Studies in Genetics,* VII. The University of Texas Publication, no. 7213: 145–153.

ROHLF, F. J., KISHPAUGH, J. & KIRK, D., 1972, *NTSYS; Numerical Taxonomy system of Multivariate Statistical Programs.* State University of New York, Stony Brook, New York. No pagination.

RYMAN, N., ALLENDORF, F. W. & STAHL, G., 1979, Reproductive isolation with little genetic divergence in sympatric populations of brown trout (*Salmo trutta*). *Genetics,* 92: 247–262.

SHAW, R. S. & PRASAD, R., 1970, Starch gel electrophoresis of enzymes; a compilation of recipes. *Biochemical Genetics,* 4: 297–320.

SNEATH, P. H. A. & SOKAL, R. R., 1973, *Numerical taxonomy: the principles and practice of numerical classification.* Freeman, San Francisco, 573 p.

TEMPLETON, A. R., 1980, Modes of speciation and inferences based on genetic distances. *Evolution,* 34: 719–729.

TRACEY, M. L., BELLET, N. F. & GRAVEN, C. D., 1975, Excess allozyme homozygosity and breeding population structure in the mussel *Mytilus californianus. Marine Biology,* 32: 303–311.

WERNER, B., 1951, Über die Bedeutung der Wasserstromerzeugung und Wasserstromfiltration für die Nahrungsaufnahme der Ortsgebunden Meeresschnecke *Crepidula fornicata* L. (Gastropoda Prosobranchia). *Zoologische Anzeiger,* 146: 97–113.

WERNER, B., 1955, Über die Anatomie, die Entwicklung, und Biologie des Veligers und der Veliconcha von *Crepidula fornicata* L. (Gastropoda Prosobranchia). *Helgoländer wissenschaftliche Meeresuntersuchungen,* 5: 169–217.

APPENDIX

Crepidula Electromorph Frequencies

Alleles given in parentheses were found in *Crepidula (Crepipatella) lingulata*, a species not included in this data set because of small sample size. These alleles are included in the tabulation of a number of alleles per locus. A zero in parentheses following the number of genome samples indicates that no results were achieved for that population at that locus. For each population, the number of genomes sampled was diminished when individuals were too small to place on every gel or when no results were forthcoming for a particular individual. Lack of results were more frequent for *C. fornicata* than the other species, perhaps becase the samples were frozen longer before using.

		Crepidula fornicata							*Crepidula convexa*			*C.* cf. *convexa*			*Crepidula plana*			*C.* cf. *plana*		*C. onyx*	*C. protea*	
Locus	Allele	1980 Bridge-port	1981 Bridge-port	M.B.L.B.	W.H.Y.C.	Mill-stone	L.C.	K.C.	Bridge-port	Mill-stone	L.C.	L.J.C.	B.H.	G.B.	Bridge-port	Mill-stone	L.C.	L.J.C.	B.H.	Balboa	Brasil	Allele
AcPh	(2N)	164	132	144	98	108	100	128	100	128	116	100	148	100	100	100	100	100	112	106	110	
	109														1.00			1.00	1.00			109
	108											1.00	1.00	1.00								108
	107															1.00	1.00					107
	105								0.94	1.00	1.00											105
	103								0.06													103
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00														100
	96																				1.00	96
	94																			1.00		94
Adkin	(2N)	164	120	116	96	100	98	128	100	116	96	100	100	100	100	98	100	100	96	104	108	
	112																			1.00		112
	109																				0.03	109
	105																				0.97	105
	102		0.03	0.02				0.02														102
	100	1.00	0.94	0.98	1.00	1.00	1.00	0.93				1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			100
	99		0.03					0.05	1.00	1.00	1.00											99
AO	(2N)	164	130	144	98	108	100	128	100	122	110	100	100	100	100	100	98	98	100	106	110	
	102																	1.00	1.00			102
	101							0.03							1.00	1.00	1.00					101
	100	0.98	1.00	0.97	0.92	1.00	1.00	0.89												1.00	1.00	100
	99	0.02		0.03	0.08			0.08	1.00	1.00	1.00	1.00	1.00	1.00								99
AAT	(2N)	164	132	144	98	104	94	128	96	120	116	100	100	100	100	100	98	98	100	106	110	
	105						0.04	0.01							0.10							105
	102		0.04	0.03	0.02			0.09							0.90	0.81	0.98	0.80	0.96			102
	101																				1.00	101
	100	1.00	0.91	0.97	0.98	1.00	0.96	0.83				0.12	0.05			0.19	0.02	0.20	0.02			100
	99								1.00	1.00	0.97	0.84	0.93	0.51								99
	98		0.05					0.07			0.03	0.04	0.02	0.49						0.16		98
	96																		0.02	0.84		96

APPENDIX (Continued)

		Crepidula fornicata							*Crepidula convexa*			*C.* cf. *convexa*			*Crepidula plana*			*C.* cf. *plana*		*C. onyx*	*C. protea*	
Locus	Allele	1980 Bridge-port	1981 Bridge-port	M.B.L.B.	W.H.Y.C.	Mill-stone	L.C.	K.C.	Bridge-port	Mill-stone	L.C.	L.J.C.	B.H.	G.B.	Bridge-port	Mill-stone	L.C.	L.J.C.	B.H.	Balboa	Brasil	Allele
EST NA I	(2N)	160	118	116	98	108	100	128	90	128	120	100	100	100	98	96	100	100	112	106	110	
	103						0.02															103
	102																				0.01	102
	101	0.05	0.03			0.06																101
	100	0.93	0.95	0.97	0.94	0.90	0.93	0.95							1.00	1.00	1.00	0.91	0.92	0.04	0.75	100
	99																			0.64		99
	98	0.02	0.02	0.03	0.06	0.04	0.03	0.05	1.00	0.99	0.87	1.00	0.95	1.00				0.09	0.08	0.31	0.24	98
	97						0.02													0.01		97
	94									0.01	0.13		0.05									94
EST NA II	(2N)	160	118	116	98	108	90	128	90	128	120	100(0)	100(0)	100(0)	98	96	100	100	112	106	110	
	103		0.05			0.05																103
	102																	1.00	1.00			102
	101	0.02	0.08	0.07	0.02	0.11	0.03													0.09		101
	100	0.90	0.79	0.86	0.96	0.77	0.80	1.00	0.50											0.91		100
	99														1.00	1.00	1.00				0.02	99
	98	0.08	0.08	0.07	0.02	0.07	0.17														0.91	98
	96																				0.07	96
	95								0.50	1.00	1.00											95
G6PD	(2N)	116	120	116	96	100	98	100	100	116	96	100	100	100	100	98	100	100	96	104	108	
	105																		0.12			105
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.87	1.00	1.00	100
	96																		0.01			96
GPI	(2N)	164	132	144	98	108	100	128	100	128	116	100	148	100	100	100	100	100	112	106	110	
	104		0.01		0.02		0.02	0.01														104
	102	0.01	0.20	0.11	0.17	0.04	0.11	0.09														102
	101			0.04		0.28	0.01			0.04	0.04											101
	100	0.89	0.67	0.66	0.44	0.54	0.77	0.87														100
	99	0.10	0.12	0.18	0.33	0.14	0.08		0.85	0.48	0.79									0.02		99
	97								0.15	0.48	0.17									0.08		97
	96			0.01	0.04		0.01	0.03														96
	95													0.10					0.05	0.23	0.01	95
	93														0.10	0.09	0.08		0.03	0.09	0.86	93
	92											1.00	1.00	0.82								92
	91														0.90	0.91	0.84	1.00	0.74	0.58	0.05	91
	88													0.08			0.06		0.14		0.08	88
	85																0.02		0.04			85

HEX	(2N)	164	120	116	94	100	96	100	100	116	96	100	100	100	98	96	100	100	96	104	108	
	111											0.37	0.15									111
	108												0.33									108
	105											0.63	0.52	1.00							1.00	105
	103								0.67	0.85	1.00											103
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.33	0.15					1.00	1.00	1.00	1.00	1.00			100
	98																			1.00		98
LDH	(2N)	164	118	116	98	108	100	98	90	122	120	100	100	100	98	96	100	100	112	106	110	
	104															0.03						104
	102														1.00	0.91	1.00					102
	100	1.0	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		0.06		1.00	1.00	1.00	1.00	100
	96		0.03																			96
LAP I	(2N)	164	132	144	98	108	100	128	100	128	116	100	148	98	100	100	100	100	112	106	110	
	106								1.00	1.00	1.00											106
	103														0.70	0.13	0.04					103
	102																	1.00	1 00			102
	101	0.02	0.02		0.03															1.00		101
	100	0 93	0.94	1.00	0.97	1.00	1.00	0.99							0.30	0.83	0.96				1 00	100
	99	0.05	0.01					0.01					0.01									99
	97		0.03									1.00	0.99	1.00		0.04						97
LAP II	(2N)	130	116	144	98	100	96	102	100	118	96	88	118	90	90	90	68	70	112	106	110	
	105								1.00	1.00	1.00										1 00	105
	103											1.00	1.00	0.99								103
	102																		0.03			102
	101							0.06						0.01						1.00		101
	100	0.96	0.87	1.00	1.00	1.00	1.00	0.94											0 02			100
	99	0.04	0.13																			99
	98														1.00	1.00	1.00	1.00	0.95			98
MPI I	(2N)	164	120	116	98	100	100	100	100	116	96	100	100	100	100	100	100	100	112	106	110	
	106		0.01			0.02																106
	105														0.22		0.07					105
	104	0.01	0.01			0.07	0.02	0.03												0.37		104
	103	0.08	0.05	0.05	0.07										0.67	0.37	0.82				1.00	103
	102								0.88	1.00	1.00									0.56		102
	101	0.01		0.02		0.04		0.04								0.58	0.11					101
	100	0.90	0.88	0.93	0.93	0.85	0.98	0.90	0.12						0.11	0.05			0.16			100
	98		0.05			0.02													0.81	0.07		98
	95							0.03				1.00	1.00	1 00				1.00	0.03			95
MPI II	(2N)	162	118	116	98	100	100	100	100	116	98	100	100	100	100	100	100	100	112	106(0)	110	
	101					0.04		0.01														101
	100	0.99	0.96	1.00	0.88	0.96	0.97	0.93														100
	99	0.01	0.02		0:12			0.06														99

APPENDIX (Continued)

Locus	Allele	*Crepidula fornicata*							*Crepidula convexa*			*C.* cf. *convexa*			*Crepidula plana*			*C.* cf. *plana*		*C. onyx*	*C. protea*	
		1980 Bridge-port	1981 Bridge-port	M.B.L.B.	W.H.Y.C.	Mill-stone	L.C.	K.C.	Bridge-port	Mill-stone	L.C.	L.J.C.	B.H.	G.B.	Bridge-port	Mill-stone	L.C.	L.J.C.	B.H.	Balboa	Brasil	Allele
MPI II	98											0.24	0.18	0.28								98
(cont.)	97		0.02				0.03		0.06	0.19	0.13			0.08					0.03		0.94	97
	96														1.00	1.00	1.00	1.00	0.97		0.06	96
	95								0.94	0.75	0.80	0.74	0.80	0.53								95
	93									0.06	0.07	0.02	0.02	0.02								93
MDH I	(2N)	164	132	144	98	108	114	128	100	122	114	100	148	96	100	100	98	100	112	106	110	
	119																			0.02		119
	114								0.13	0.06										0.96		114
	111								0.54	0.76	0.81							0.50	0.07	0.02		111
	109.5											1.00	0.97	1.00								109.5
	109			0.01																		109
	108								0.21	0.16	0.17				0.90	1.00	1.00	0.50	0.92			108
	107												0.03						0.01			107
	106				0.02			0.01	0.12	0.02	0.02				0.10						0.01	106
	104			0.01			0.01	0.04													0.99	104
	100	0.98	0.98	0.96	0.98	1.00	0.99	0.94														100
	98		0.01																			98
	96	0.02	0.01	0.02				0.01														96
MDH II	(2N)	150	132	144	90	108	114	88	102	102	116(0)	90	120	92	90	98	82	92	104	100	110(0)	
	108	0.07																				108
	102		0.04	0.12		0.18						1.00	1.00	1.00						0.02		102
	101		0.02			0.04																101
	100	0.87	0.93	0.80	1.00	0.78	1.00	0.97												0.88		100
	99																			0.09		99
	98	0.05	0.01	0.08											1.00	1.00	0.93	1.00	0.61	0.01		98
	97								0.17	0.17									0.09			97
	96	0.01						0.02									0.07					96
	95								0.83	0.83												95
	93																		0.30			93
	90							0.01														90

Pep T I	(2N)	116	124	116	96	100	114	128	100	128	116	100	100	100	100	100	100	100	112	106	110	
	107									0.02	0.06											107
	106								1.00	0.95	0.94											106
	104									0.03		0.14	0.14								0.05	104
	103		0.01			0.02																103
	102														0.43				0.04		0.94	102
	101		0.04			0.04	0.01	0.03						0.18							0.01	101
	100	0.99	0.92	1.00	0.98	0.94	0.99	0.97						0.03								100
	99	0.01	0.03		0.02							0.68	0.50	0.75	0.43	1.00	0.23	0.21	0.34			99
	98											0.18	0.33	0.04	0.14		0.77	0.72	0.46			98
	96												0.03					0.07	0.16	1.00		96
Pep T II	(2N)	116	124	116	96	100	114	128	100	128	116	100	100	100	100	100	100	100	112	106	110	
	103																				1.00	103
	(102)																					(102)
	100	1.00	1.00	1.00	0.91	1.00	1.00	0.97							1.00	1.00	1.00	1.00	0.71			100
	99				0.09			0.03												0.93		99
	98												0.05	0.02					0.06	0.07		98
	97																		0.23			97
	96								1.00	1.00	1.00	1.00	0.80	0.91								96
	94												0.15	0.07								94
Pep G III	(2N)	116	124	116	96	100	114	128	100	128	116	100	100	100	92	100	100	102	100	100	110	
	105					0.02						1.00	1.00	0.96			0.04		0 25			105
	103									0.16				0 04				0.07	0 51			103
	102		0 04		0.24	0.17	0.02										0.14					102
	100	0.80	0.83	0.89	0.62	0.61	0.87	0.98	1.00	0.67	1.00				1.00	1.00	0.50	0 93	0.24		0 02	100
	99	0.04	0.02		0.04					0.17												99
	98																0.32				0.02	98
	97	0.16	0.11	0.11	0.10	0.20	0.11	0.02													0.96	97
	93																			1.00		93
PGM	(2N)	160	118	116	98	108	100	128	100	128	120	100	100	100	100	94	100	100	112	106	110	
	102	0.03	0.10	0.13	0.19	0.11	0.02	0.06							0.08					0 02		102
	100	0.95	0.81	0.80	0.72	0.84	0.92	0.79												0.95	0 03	100
	99										0.07			0.02			0.05		0.02		0 05	99
	98	0.02	0.09	0.03	0.09	0.05	0.03	0.11			0.02		0.03	0.22	0.50	0.92	0.64	0 71	0 82	0.03	0 25	98
	97			0.04			0.03	0.04	0.91	0.85	0.91	0.96	0.85	0.75							0 64	97
	96											0.04	0.12	0.01					0.10			96
	95								0.09	0.15					0.25	0.08	0.27				0 03	95
	94														0.17		0.04	0.29	0.06			94

APPENDIX (Continued)

		Crepidula fornicata							*Crepidula convexa*			*C.* cf. *convexa*			*Crepidula plana*			*C.* cf. *plana*		*C. onyx*	*C. protea*	
Locus	Allele	1980 Bridge-port	1981 Bridge-port	M.B.L.B.	W.H.Y.C.	Mill-stone	L.C.	K.C.	Bridge-port	Mill-stone	L.C.	L.J.C.	B.H.	G.B.	Bridge-port	Mill-stone	L.C.	L.J.C.	B.H.	Balboa	Brasil	Allele
6PGDH	(2N)	116	124	116	96	100	114	128	100	128	116	100	100	100	100	100	100	100	100	106	110	
	110									0.16												110
	107									0.41												107
	105								0.40	0.40												105
	103																			1.00	1.00	103
	102								0.40	0.04	0.93											102
	101	0.22	0.04																			101
	100	0.75	0.88	1.00	1.00	1.00	1.00	1.00	0.20		0.07				1.00	1.00	1.00	1.00	1.00			100
	98	0.03	0.08									1.00	0.98	1.00								98
	95												0.02									95
SDH	(2N)	164	130	144	98	108	100	128	100	122	110	100	100	100	100	100	100	100	100	106	110	
	102	0.13	0.12	0.03		0.12																102
	100	0.87	0.88	0.97	1.00	0.88	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00		0.14						100
	99							0.03														99
	98														1.00	0.86	1.00	1.00	1.00	0.11		98
	97																				1.00	97
	95																			0.89		95
SoDH	(2N)	164	132	144	98	108	100	128	100	122	110	100	100	100	100	100	100	100	100	106	110	
	(106)																					(106)
	104																				1.00	104
	103		0.01							0.02												103
	102		0.01					0.01														102
	101																	1.00	1.00			101
	100	1.00	0.98	1.00	1.00	1.00	1.00	0.99	1.00	0.98	1.00	1.00	1.00	1.00		0.04						100
	99															0.08				1.00		99
	98														1.00	0.88	1.00					98
XDH	(2N)	164	120	116	96	100	98	128	100	116	100	100	100	100	100	100	100	100	96	106	110	
	101		0.03																			101
	100	0.97	0.93	0.95	1.00	0.90	1.00	0.99														100
	99														1.00	1.00	1.00	1.00	1.00	1.00	1.00	99
	98	0.03	0.04	0.05		0.10		0.01	1.00	1.00	1.00	1.00	1.00	1.00								98

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GENETIC RELATIONSHIPS AMONG SOME NORTH AMERICAN UNIONIDAE (BIVALVIA): SIBLING SPECIES, CONVERGENCE, AND CLADISTIC RELATIONSHIPS

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ABSTRACT

Allozyme analyses involving 14 loci were used to assess the molecular genetic relationships among 39 populations representing 25+ species of unionid clams. Of particular concern were the relationships between genera within and between the endemic North American tribes Amblemini and Pleurobemini of the subfamily Ambleminae. The distribution of species per genus in the Pleurobemini was: *Elliptio* (14+), *Fusconaia* (2), *Uniomerus* (3); in the Amblemini: *Megalonaias* (1), *Quadrula* (1). The distribution of species per genus of uncertain tribal placement was: *Elliptoideus* (1), *Quincuncina* (1). The out-group comparator was *Lampsilis* (1) of the tribe Lampsilini. A matrix of Nei's genetic distances was used in multivariate analyses to produce a two-dimensional diagram of OTU projections on the first two principal components following 3-D scaling; a Prim Network was used.

The purposes of these analyses were: 1) to determine the relationships among species of *Elliptio* including several populations of lanceolate taxa with different shell phenotypes; 2) to determine the relationships beween *Fusconaia*, *Uniomerus* and *Elliptio*; 3) to determine the relationships of *Elliptoideus* and *Quincuncina* to genera assigned to the tribes Pleurobemini and Amblemini (in Davis & Fuller, 1981), because of uncertainty of these relationships following immunoelectrophoretic studies (Davis & Fuller, 1981). Individual heterozygosity (H) and frequencies of polymorphism (P) were assessed in relationship to species and higher taxa.

Uniomerus is divergent from *Elliptio* yet clearly in the same tribe, the Pleurobemini. The amount of genetic divergence among species of *Uniomerus* approximates the greatest divergence among species of *Elliptio*. *Elliptoideus* and *Quincuncina* group with other genera of the Amblemini. There appears to have been parallel evolution of lanceolate *Elliptio* giving rise to three separate clades. The number of species with lanceolate shells has recently been considerably underestimated. The greatest genetic distance between species of *Elliptio* is between lanceolate taxa (D = 0.732) of which there are at least seven species. *Fusconaia succissa* and *F. flava* clearly belong in different genera.

Genetic differences among genera of the Amblemini (D= 0.651 ± 0.275) are considerably greater than those among genera of the Pleurobemini; the variances are also considerably greater. Genetic differences between genera of the Pleurobemini (D = 0.243 ± 0.086) are of a level attributed to differences between unionid congeneric species. It is probable that genera of the Pleurobemini are more recently evolved than those of the Amblemini considering the lower genetic distance and lower genetic variance among genera of the Pleurobemini. The highest values of P and H are associated with the Pleurobemini and especially non-lanceolate *Elliptio*.

Two cladograms are presented. One is based on anatomical data consistent with immunological data, biogeography, and paleontological data. The other adjusts branching sequences of the first cladogram to be consistent with age of divergence assuming that the greater the genetic distance, the greater the age of divergence.

Key words: genetics; molecular genetics; allozymes; Unionidae; North America; systematics; convergence; evolution; multivariate analysis.

INTRODUCTION

The Unionidae comprise a freshwater bivalve assemblage that exceeds 45 nominal genera and includes some 225 species and subspecies currently recognized in North America alone (Burch, 1973, 1975). Several genera within the Unionidae contain more than seven species. Some genera have 10 or more species. Such large first-order radia-

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tions (Davis 1981a,b) are brought to the attention of students of evolution interested in tempos and modes, patterns and processes of speciation for the following reasons: unionids are an ancient group, pre-dating the Upper Cretaceous. While some clades are widespread on northern continents and Africa, others are largely or wholly endemic in North America. Some clades are apparently decreasing in diversity (e.g. Anodontinae; Davis & Fuller, 1981).

Studies of unionids have long been hindered by lack of an adequate species concept. The biological species concept is not suitable because one cannot particularly attempt crosses between populations in the laboratory to test for reproductive compatibility. Unionids take too long to mature and their life cycle depends on glochidial attachment on fish, often involving considerable host specificity. The species concept that has been most frequently used is based almost entirely on conchology. The morphological variance within and among populations and species is so great that it is frequently difficult to distinguish species on the basis of conchology (Davis *et al.,* 1981; Kat, 1983d; Davis, 1983). Species recognition is further hampered by conchological convergences and parallelisms. These convergences and parallelisms are presumably due to the limited possible number of shell morphologies for freshwater bivalves restricted to limited substrate types and other microhabitat variables. One must also consider the following as they relate to speciation: the ephemeral nature of freshwater bodies, mountain orogenies and the evolution of river systems *including* stream capture, glaciation and sea level fluctuations, and the coevolution of fish and the parasitic unionid glochidial larvae (Kat, 1984; Kat & Davis, in press).

Given the problems with conchological analyses, one does not know the extent to which the number of species of a given genus has been over- or under-estimated. Recently, it was shown that the number of species of *Elliptio* and *Uniomerus* was underestimated because of convergence and the existence of cryptic species (i.e. sibling species; Davis *et al.,* 1981; Davis, 1983).

Elliptio and *Uniomerus* are members of the subfamily Ambleminae, tribe Pleurobemini (Davis & Fuller, 1981). Understanding of species and genera of this tribe has been especially confused because of extreme conchological variability and purported lack of anatomical differences between species. Additionally, it is not clear whether certain nominal genera are members of the Pleurobemini or of a closely related clade, the Amblemini. In this paper allozyme analyses are used to assess the molecular genetic relationships among 39 populations belonging to 24+ species. The purposes of these analyses are: 1) to assess the relationships among 14+ species of *Elliptio*; 2) to examine the relationship between *Uniomerus* and *Elliptio*. How much genetic divergence is there between these purportedly closely related genera relative to other genera of the tribes Pleurobemini and Amblemini? What are the average maximum amounts of genetic distance between species of *Uniomerus* compared with those between species of *Elliptio*? 3) to determine the relationships of *Elliptoideus* and *Quincuncina* to genera of the tribes Amblemini and Pleurobemini *sensu* Davis & Fuller (1981). There were insufficient data to do this immunologically in Davis & Fuller (1981). 4) The data are further used to assess cladistic relationships proposed earlier (Davis & Fuller, 1981; Davis *et al.,* 1981).

MATERIALS AND METHODS

Taxa studied

An alphabetical listing of taxa studied is given in Table 1 along with the code for each population, the Academy of Natural Sciences (ANSP) voucher catalog number, locality and date collected. There are 39 populations of eight genera and 24+ species. Representative shells are shown in Figs. 1, 2.

Electrophoresis and analysis of data

Horizontal starch gel electrophoresis and methods of data analysis were used as described in Davis *et al.* (1981). Fourteen loci were resolved, Nei's (1972) D and I statistics were calculated on the basis of allele frequencies ≥ 0.04. Mean individual heterozygosity (H) and percentage of polymorphic loci (P) were calculated. The matrix of D statistics was used as a starting point for multivariate analysis yielding two-dimensional ordination diagrams following three-dimensional scaling. The Prim Network was used.

TABLE 1. Taxa studied, listed with code, ANSP catalog number, locality, and date of collection.

1.	Ea[2]	*Elliptio arctata* (Conrad); 348882; N. Mosquito Creek (E. channel), Gadsden Co., Florida; October, 1978.
2.	Eb[2]	*E. buckleyi* (Lea); 342285; St. Johns River at Rt. 17 crossing, Lake Monroe, Volusia Co., Florida; January, 1977.
3.	Eci	*E. cistelliformis* (Lea); 345064; Lake Waccamaw, Waccamaw River drainage, Columbus Co., North Carolina; August, 1977.
4.	Ec[6-3]	*E. complanata* (Lightfoot); 349333; Deep Creek, Nanticoke River drainage, Sussex Co., Delaware; June, 1978.
5.	Ec[8-2]	*E. complanata*; 352824; Swartswood Lake, Delaware River drainage, Sussex Co., New Jersey; May, 1980.
6.	Ec[9]	*E. complanata*; 341950; Kennebec River, Somerset Co., Maine; August, 1976.
7.	Ec[11]	*E. complanata*; 345055; Chester River drainage, Queen Annes Co., Maryland; May, 1977.
8.	Ec[12]	*E. complanata*; 345056; Sassafras River, Kent Co., Maryland; May, 1977.
9.	Ec[22]	*E. complanata*; 352832; Tar River, 0.8 km E. of U.S. Route 1; Franklin-Vaner Co., about 6 km N. of Route 1, Franklinton, Franklin Co., North Carolina; June, 1980.
10.	Ec[23]	*E. complanata*; 353127; Buckhead Creek, Magnolia Springs, Ogeechee River drainage, Jenkins Co., Georgia; June, 1980.
11.	Ec[28]	*E. "complanata"*; 353256; Savannah River. Allendale Co., South Carolina; September, 1980.
12.	Eco[3]	*E. congaraea* (Lea); 353255; as for Ec[28].
13.	Ecr[4A]	*E. crassidens* (Lamarck); 348880; Apalachicola River, Gadsden Co., Florida; October, 1978.
14.	Ecr[4B]	*E. crassidens*; 348876; as for Ecr[4A].
15.	Ecr[5]	*E. crassidens*; 349025; Conecuh River; Alabama Route 41; Escambia Co., Alabama; October, 1978.
16.	Ed[11]	*E. dilatata* (Rafinesque); 353171; New River at Allisonia; Pulaski Co., Virginia; July, 1980.
17.	Efi	*E. fisheriana* (Lea); 349337; Concord Pond, Nanticoke River drainage, Sussex Co., Delaware; June, 1978.
18.	Efo	*E. folliculata* (Lea); 352604; Lake Waccamaw, Waccamaw River drainage, Columbus Co., North Carolina; August 1977.
19.	Ei[17]	*E. icterina* (Conrad); topotypes; 353253; Savannah River, Allendale Co., South Carolina; September, 1980.
20.	El[6]	*E. producta* (Conrad); 352537; as for Efo.
21.	El[7]	*E. lanceolata* (Lea); topotypes; 352833; Tar River, Franklin Co., North Carolina; June, 1980.
22.	El[8]	*E.* sp.; 353128; as for Ec[23].
23.	El[9]	*E.* sp.; 353129; as for Ec[23].
24.	El[10]	*E.* sp.; 353254; as for Ei[17].
25.	El[11]	*E.* sp.; 353265; Fountain's Mill, Ochmulgee River drainage, Pulaski Co., Georgia; September, 1980.
26.	Em[1]	*E. mcmichaeli* Clench & Turner; 349038; Pea River, Geneva Co., Alabama; October, 1978.
27.	Em[2]	*E. mcmichaeli*; 348858; Choctawhatchee River, Washington Co., Florida; October, 1978.
28.	Es[2]	*E. shepardiana* (Lea); 342972; Ochmulgee River; Coffee-Ben Hill Cos., Georgia; September, 1980.
29.	Ew	*E. waccamawensis* (Lea); 345061; as for Efo.
30.	Esl[2]	*Elliptoideus sloatianus* (Lea); 348838; Ochlockonee River, Leon Co., Florida; October, 1978.
31.	Ff[3]	*Fusconaia flava* (Rafinesque); 350060; Upper Mississippi River, Vernon Co., Wisconsin; June, 1979.
32.	Fs	*F. succissa* (Lea); 349018; Escambia River, Escambia Co., Florida; October, 1978.
33.	Lt[3]	*Lampsilis teres* (Rafinesque); 348872; Apalachicola River, Gadsden Co., Florida; October, 1978.
34.	Mb[3]	*Megalonaias boykiniana* (Lea); 348842; as for Esl[2].
35.	Qq[1]	*Quadrula quadrula* (Rafinesque); 350057; as for Ff[3].
36.	Qi[3]	*Quincuncina infucata* (Conrad); 348844; as for Esl[2].
37.	Ue	*Uniomerus excultus* (Conrad); 353133; as for Ec[23].
38.	Ud	*U. declivis* (Say); 350750; 350751; as for Uc.
39.	Uc	*U. carolinianus* (Bosc); 350750, 350751; Mosquito Creek; E. branch (center and banks); Gadsden Co., Florida; September, 1979.

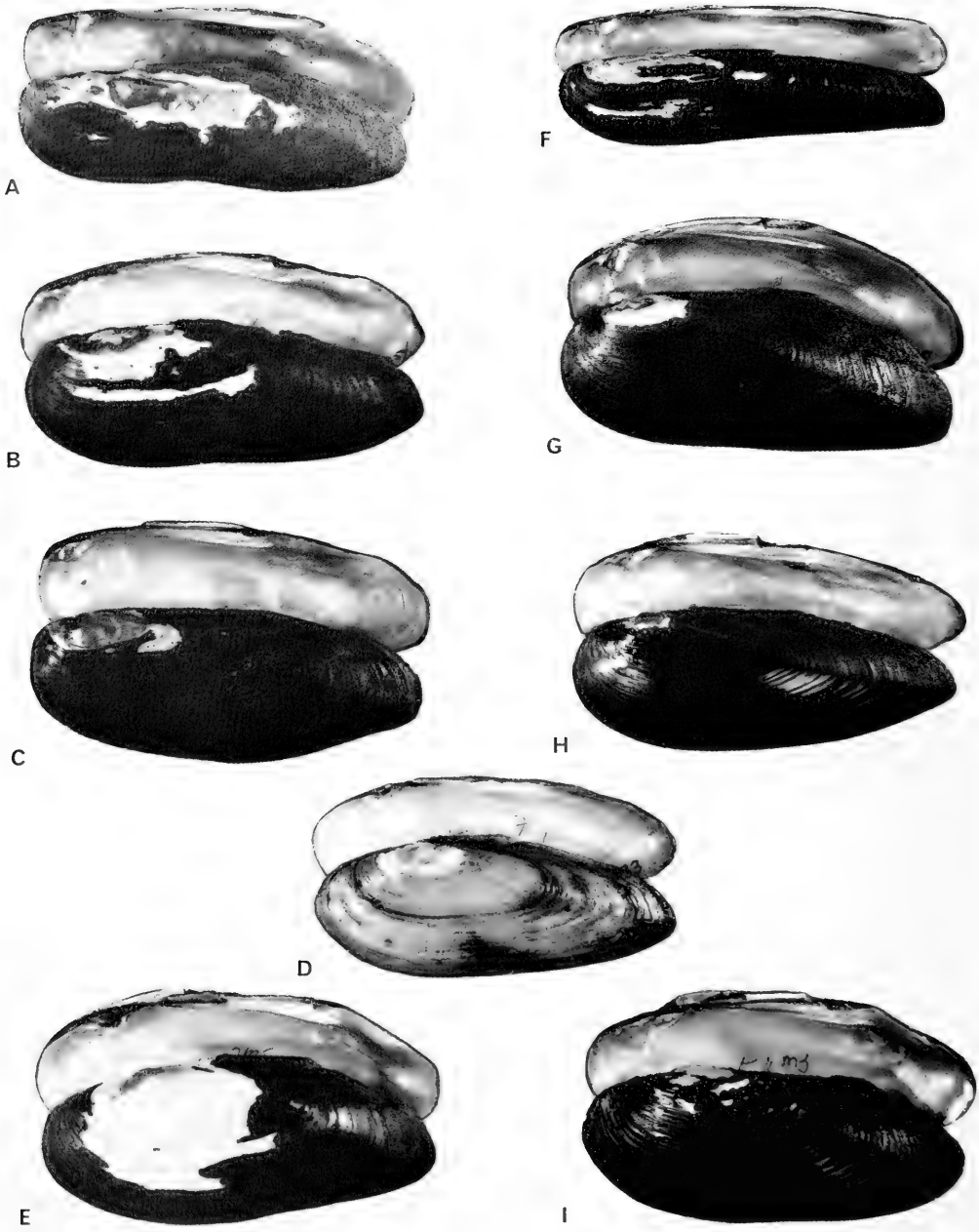

FIG. 1. Shells of lanceolate *Elliptio*. Refer to Table 1 for localities. Shell length in mm ( ). A. *E. folliculata*; Efo, No. 18 (81.5). B. El[10], No. 24 (98.5). C. *E. producta*; El[6], No. 20 (72.0). D. *E. lanceolata* topotype; El[7], No. 21 (57.5). E. El[9], No. 23 (111.0). F. *E. shepardiana*; Es[2], No. 28 (170.0). G. El[11], NO. 25 (76.5). H. *E. fisheriana*; Efi, No. 17 (72.5). I. El[8], No. 22 (83.0).

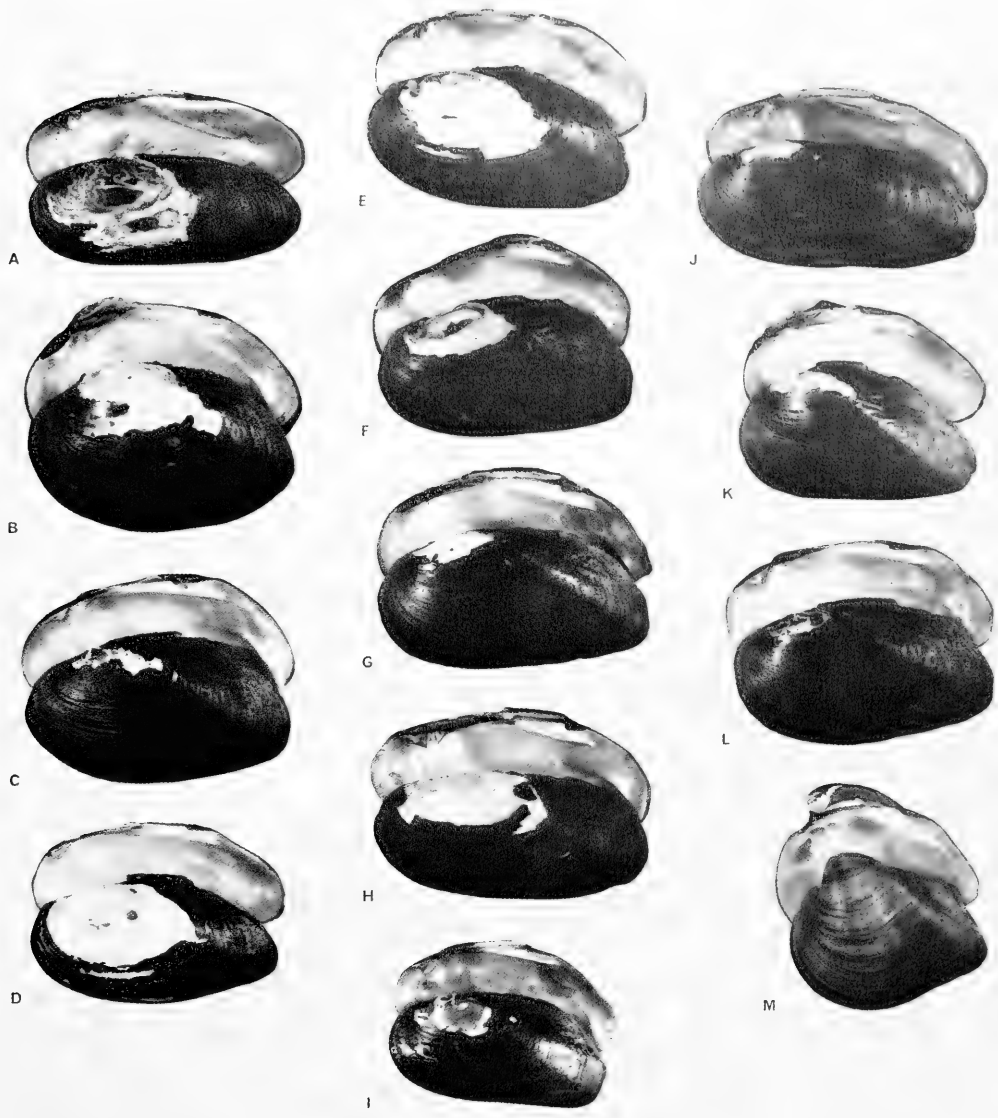

FIG. 2. Shells of the ovate-quadrate group of *Elliptio* contrasted with triangular *Fusconaia*. Refer to Table 1 for localities. Shell length in mm ( ). A. *E. arctata*; Ea[2], No. 1 (104.5). B. *E. congaraea*; Eco[3], No. 12 (89.5). C. *E. complanata*; Ec[11], No. 7 (89.0). D. *E. buckleyi*; Eb[2], No. 2 (57.0). E. *E. icterina*; topotype, Ei[17], No. 19 (75.0). F. *E. mcmichaeli*; Em[2], No. 27 (76.5). G. *E. crassidens*. Ecr[5], No. 15 (81.0). H. *E. "complanata"*; Ec[28], No. 11 (125.5). I. *E. cistelliformis*; Eci, No. 3 (52.0). J. *E. dilatata*; Ed[11], No. 16 (81.0). K. *E. waccamawensis*; Ew, No. 29 (60.0). L. *E. complanata*; Ec[6], No. 4 (90.0). M. *Fusconaia flava*; Ff[3], No. 31 (72.5).

TABLE 2. Nei's D (above diagonal) and I (below diagonal) for 39 populations.Codes explained in Table 1.

	Ea[2]	Eb[2]	Eci	Ec[6]	Ec[8]	Ec[9]	Ec[11]	Ec[12]	Ec[22]	Ec[23]	Ec[28]	Eco[3]	Ecr[4A]	Ecr[4B]	Ecr[5]	Ed[11]	Efi	Efo	Ei[17]	El[6]
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.
1.	—	.089	.085	.055	.088	.103	.084	.110	.050	.090	.128	.166	.092	.079	.075	.143	.324	.132	.056	.217
2.	.918	—	.046	.051	.036	.024	.025	.070	.061	.047	.129	.205	.093	.110	.126	.166	.389	.160	.099	.307
3.	.919	.955	—	.033	.077	.089	.069	.098	.068	.097	.144	.183	.067	.071	.081	.163	.359	.182	.082	.292
4.	.946	.950	.968	—	.061	.071	.046	.078	.051	.072	.099	.148	.067	.069	.070	.132	.290	.118	.061	.217
5.	.916	.965	.926	.941	—	.033	.032	.070	.037	.037	.102	.225	.122	.137	.147	.167	.348	.177	.063	.303
6.	.902	.976	.915	.931	.968	—	.023	.044	.074	.034	.124	.235	.102	.124	.141	.202	.433	.156	.116	.340
7.	.919	.975	.933	.955	.969	.977	—	.062	.060	.033	.113	.201	.100	.116	.123	.182	.371	.151	.106	.308
8.	.896	.932	.907	.925	.932	.957	.940	—	.074	.083	.137	.252	.112	.131	.142	.192	.422	.133	.131	.367
9.	.951	.940	.934	.950	.964	.929	.942	.929	—	.066	.105	.193	.120	.122	.123	.139	.325	.150	.041	.306
10.	.914	.954	.908	.931	.964	.967	.968	.920	.936	—	.116	.217	.117	.126	.133	.216	.381	.202	.113	.310
11.	.880	.879	.866	.906	.903	.883	.893	.872	.900	.890	—	.178	.098	.101	.093	.177	.400	.109	.080	.258
12.	.847	.815	.833	.862	.799	.791	.818	.777	.824	.805	.837	—	.150	.136	.105	.258	.379	.186	.156	.315
13.	.912	.911	.935	.935	.885	.903	.905	.894	.887	.890	.907	.861	—	.005	.018	.183	.416	.103	.080	.237
14.	.924	.896	.931	.933	.872	.883	.890	.877	.885	.882	.904	.873	.995	—	.008	.179	.411	.104	.074	.219
15.	.928	.882	.922	.932	.863	.868	.884	.868	.884	.875	.911	.900	.982	.992	—	.194	.387	.096	.069	.227
16.	.867	.847	.850	.876	.846	.817	.834	.825	.870	.806	.838	.773	.833	.836	.824	—	.355	.242	.134	.268
17.	.723	.678	.698	.748	.706	.649	.690	.656	.723	.683	.670	.685	.660	.663	.679	.701	—	.433	.313	.222
18.	.876	.852	.833	.889	.838	.856	.860	.875	.861	.817	.897	.830	.902	.901	.908	.785	.649	—	.117	.230
19.	.946	.906	.921	.941	.939	.890	.899	.877	.960	.893	.923	.856	.923	.929	.933	.875	.731	.890	—	.225
20.	.805	.736	.747	.805	.739	.712	.735	.693	.737	.733	.773	.730	.789	.803	.797	.765	.801	.795	.799	—

D Statistic only for 1–20 × 21–39

	El[7]	El[8]	El[9]	El[10]	El[11]	Em[1]	Em[2]	Es[2]	Ew	Esl[2]	Ff[3]	Fs	Lt[3]	Mb[3]	Qq[1]	Qi[3]	Ue	Ud	Uc
	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.	39.
1.	.373	.199	.143	.219	.297	.103	.086	.356	.066	.515	.228	.440	.731	.697	.834	.569	.217	.340	.503
2.	.293	.084	.095	.205	.281	.126	.101	.277	.054	.661	.254	.533	.842	.780	.804	.618	.292	.421	.582
3.	.393	.166	.122	.270	.333	.103	.084	.383	.015	.675	.313	.519	.846	.753	.895	.563	.276	.403	.569
4.	.331	.147	.119	.213	.270	.094	.074	.313	.016	.589	.239	.473	.843	.670	.703	.585	.216	.337	.459
5.	.277	.097	.118	.220	.233	.139	.118	.291	.069	.620	.219	.462	.851	.785	.715	.568	.291	.421	.550
6.	.242	.040	.099	.181	.210	.142	.119	.215	.092	.643	.197	.507	.807	.818	.682	.589	.274	.398	.542
7.	.248	.084	.099	.207	.240	.128	.113	.263	.058	.596	.184	.502	.801	.729	.695	.574	.287	.375	.576

8.	.291	.059	.123	.251	.256	.172	.127	.281	.095	.502	.238	.399	.821	.787	.634	.497	.232	.350	.469
9.	.321	.140	.118	.258	.293	.133	.107	.365	.050	.584	.324	.450	.841	.676	.805	.608	.257	.392	.493
10.	.224	.086	.125	.194	.228	.152	.139	.278	.086	.680	.202	.549	.794	.839	.690	.619	.304	.429	.578
11.	.329	.196	.177	.185	.212	.098	.088	.239	.140	.609	.241	.477	.965	.735	.715	.579	.308	.454	.485
12.	.499	.317	.237	.270	.343	.100	.107	.409	.169	.768	.354	.633	.839	.595	.606	.642	.365	.316	.595
13.	.346	.172	.133	.180	.216	.042	.038	.222	.087	.583	.188	.501	.797	.787	.807	.510	.249	.385	.507
14.	.365	.203	.140	.180	.223	.038	.037	.248	.090	.574	.199	.504	.785	.769	.838	.518	.252	.386	.514
15.	.377	.234	.150	.184	.228	.031	.035	.273	.093	.580	.217	.512	.771	.693	.802	.524	.257	.361	.492
16.	.532	.244	.207	.342	.365	.203	.156	.460	.141	.635	.391	.463	1.017	.830	.850	.682	.223	.368	.444
17.	.501	.528	.479	.591	.699	.419	.367	.732	.264	.877	.589	.658	1.097	.946	.828	.853	.478	.434	.601
18.	.308	.252	.186	.180	.271	.096	.086	.227	.174	.518	.253	.508	.915	.664	.844	.635	.324	.421	.548
19.	.380	.217	.146	.218	.232	.061	.053	.328	.073	.577	.276	.441	.846	.676	.818	.558	.228	.369	.437
20.	.385	.451	.373	.320	.433	.225	.208	.466	.238	.616	.306	.542	1.019	.983	.904	.757	.357	.393	.668

I Statistic for Taxa 21–39 × 1–20

	Ea^{2}	Eb^{2}	Eci	Ec^{6}	Ec^{8}	Ec^{9}	Ec^{11}	Ec^{12}	Ec^{22}	Ec^{23}	Ec^{28}	Eco^{3}	Ecr^{4A}	Ecr^{4B}	Ecr^{5}	Ed^{11}	Efi	Efo	Ei^{17}	El^{6}
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.
21.	.689	.746	.675	.718	.758	.785	.780	.748	.725	.799	.720	.607	.708	.694	.686	.587	.606	.735	.684	.680
22.	.820	.919	.847	.863	.908	.961	.919	.943	.869	.918	.822	.728	.842	.816	.791	.783	.590	.777	.805	.637
23.	.867	.909	.885	.888	.889	.906	.906	.884	.889	.882	.838	.789	.875	.869	.861	.813	.619	.830	.864	.689
24.	.803	.815	.763	.808	.802	.834	.813	.778	.773	.824	.831	.763	.835	.835	.832	.710	.554	.835	.804	.726
25.	.743	.755	.717	.763	.792	.810	.787	.774	.746	.796	.809	.710	.806	.800	.796	.694	.497	.763	.793	.649
26.	.902	.882	.902	.910	.870	.868	.880	.842	.875	.859	.910	.905	.959	.963	.969	.816	.658	.908	.941	.799
27.	.918	.904	.919	.929	.889	.888	.893	.881	.899	.870	.916	.899	.963	.964	.966	.856	.693	.918	.948	.812
28.	.700	.759	.682	.731	.747	.807	.769	.755	.694	.757	.787	.664	.801	.780	.761	.631	.481	.797	.720	.628
29.	.936	.947	.985	.984	.933	.912	.944	.909	.951	.918	.869	.845	.917	.914	.911	.868	.768	.840	.930	.788
30.	.598	.516	.509	.555	.538	.526	.551	.605	.558	.507	.544	.464	.558	.563	.560	.530	.416	.596	.562	.540
31.	.796	.776	.731	.787	.803	.821	.832	.788	.723	.817	.786	.702	.829	.820	.805	.676	.555	.776	.759	.736
32.	.644	.587	.595	.623	.630	.602	.605	.671	.638	.578	.621	.531	.606	.604	.599	.629	.518	.602	.643	.582
33.	.481	.431	.429	.430	.427	.447	.449	.440	.431	.452	.381	.432	.451	.456	.463	.362	.334	.401	.429	.361
34.	.498	.458	.471	.512	.456	.441	.482	.455	.509	.432	.480	.552	.455	.463	.500	.436	.388	.515	.509	.374
35.	.434	.448	.409	.495	.489	.506	.499	.530	.447	.502	.489	.546	.446	.433	.448	.427	.437	.430	.441	.405
36.	.566	.539	.569	.557	.567	.555	.563	.608	.544	.538	.560	.526	.600	.596	.592	.506	.426	.530	.572	.469
37.	.805	.747	.759	.806	.748	.760	.751	.793	.773	.738	.735	.694	.780	.777	.773	.800	.620	.723	.796	.700
38.	.712	.656	.668	.714	.656	.672	.687	.705	.676	.651	.635	.729	.680	.680	.697	.692	.648	.656	.691	.675
39.	.605	.559	.566	.632	.577	.582	.562	.626	.611	.561	.616	.552	.602	.598	.611	.641	.548	.578	.646	.513

TABLE 2 (Continued) D and I Statistics for Taxa 21–39 thus finishing the matrix

	El[7]	El[8]	El[9]	El[10]	El[11]	Em[1]	Em[2]	Es[2]	Ew	Esl[2]	Ff[3]	Fs	Lt[3]	Mb[3]	Qq[1]	Qi[3]	Ue	Ud	Uc
	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37.	38.	39.
21.	—	.242	.304	.371	.437	.382	.370	.362	.348	.675	.287	.624	.989	.864	.737	.635	.536	.522	.780
22.	.785	—	.129	.251	.255	.237	.185	.248	.169	.613	.245	.454	.889	.976	.581	.538	.256	.398	.481
23.	.738	.879	—	.274	.321	.158	.136	.327	.130	.521	.301	.528	.932	.665	.807	.603	.279	.396	.388
24.	.690	.778	.760	—	.077	.180	.172	.062	.279	.801	.257	.693	1.066	1.013	.938	.839	.436	.595	.710
25.	.646	.775	.725	.926	—	.207	.213	.105	.344	.731	.230	.660	1.045	1.116	.743	.679	.390	.539	.638
26.	.682	.789	.854	.835	.813	—	.014	.273	.116	.573	.210	.511	.807	.689	.778	.511	.260	.341	.541
27.	.691	.831	.873	.842	.808	.986	—	.261	.092	.523	.210	.431	.822	.706	.763	.500	.205	.313	.454
28.	.696	.780	.721	.940	.900	.761	.770	—	.402	.820	.258	.729	1.154	1.164	.903	.812	.509	.679	.715
29.	.706	.845	.878	.757	.709	.890	.912	.669	—	.628	.301	.483	0.835	.695	.783	.578	.243	.330	.517
30.	.509	.542	.594	.449	.481	.564	.593	.440	.534	—	.446	.375	1.391	.634	.686	.358	.478	.564	.645
31.	.751	.783	.740	.773	.795	.811	.811	.773	.740	.640	—	.468	.774	1.050	.643	.437	.341	.428	.705
32.	.536	.635	.590	.500	.517	.600	.650	.482	.617	.687	.626	—	1.263	1.030	.590	.417	.345	.483	.575
33.	.372	.411	.394	.344	.352	.446	.440	.315	.434	.249	.461	.283	—	1.510	1.480	1.215	.915	.823	1.457
34.	.421	.377	.514	.363	.328	.502	.494	.312	.499	.530	.350	.357	.221	—	.935	.969	.890	.781	.945
35.	.479	.559	.446	.391	.476	.459	.466	.405	.457	.504	.526	.554	.228	.393	—	.321	.602	.521	.716
36.	.530	.584	.547	.432	.507	.600	.606	.444	.561	.699	.646	.659	.297	.379	.725	—	.476	.458	.722
37.	.585	.774	.757	.647	.677	.771	.815	.601	.784	.620	.711	.708	.401	.411	.548	.621	—	.216	.201
38.	.593	.672	.673	.552	.583	.711	.731	.507	.719	.569	.652	.617	.439	.458	.594	.633	.806	—	.484
39.	.458	.618	.678	.492	.528	.582	.635	.489	.596	.525	.494	.563	.233	.389	.489	.486	.812	.616	—

TABLE 3. Average individual heterozygosity (H), % of loci that are polymorphic (P), and number of individuals (N) used for each of 39 populations. Codes explained in Table 1.

Population	H	P	N	Population	H	P	N
1. Ea[2]	0.112	0.429	35	21. El[7]	0.021	0.143	17
2. Eb[2]	0.105	0.285	28	22. El[8]	0.120	0.285	24
3. Eci	0.080	0.357	25	23. El[9]	0.173	0.500	13
4. Ec[6-3]	0.161	0.500	25	24. El[10]	0.122	0.357	26
5. Ec[8-2]	0.128	0.500	28	25. El[11]	0.099	0.285	24
6. Ec[9]	0.132	0.500	25	26. Em[1]	0.150	0.500	18
7. Ec[11]	0.129	0.500	25	27. Em[2]	0.170	0.500	27
8. Ec[12]	0.122	0.642	25	28. Es[2]	0.077	0.214	15
9. Ec[22]	0.119	0.285	25	29. Ew	0.099	0.357	30
10. Ec[23]	0.159	0.429	18	30. Esl[2]	0.082	0.214	21
11. Ec[28]	0.214	0.429	12	31. Ff[3]	0.107	0.285	16
12. Eco[3]	0.187	0.357	22	32. Fs	0.081	0.429	25
13. Ecr[4A]	0.130	0.429	25	33. Lt[3]	0.056	0.285	25
14. Ecr[4B]	0.170	0.429	25	34. Mb[3]	0.022	0.143	30
15. Ecr[5]	0.212	0.500	26	35. Qq[1]	0.112	0.357	20
16. Ed[11]	0.106	0.285	23	36. Qi[3]	0.083	0.285	25
17. Efi	0.085	0.285	15	37. Ue	0.111	0.429	18
18. Efo	0.100	0.285	13	38. Ud	0.119	0.214	3
19. Ei[17]	0.188	0.500	17	39. Uc	0.086	0.214	5
20. El[6]	0.049	0.143	16				

TABLE 4. Comparisons of unionid taxa involving the mean and standard deviation of P and H. Abbreviations for some taxa are given in Table 1. N = number of populations unless otherwise stated. S = sample size per population.

	No.	S	P	H
Ambleminae				
Pleurobemini				
1. *Elliptio complanata* (Ec)	8	24 ± 3	.47 ± .10	.146 ± .032
2. Other non-lanceolate *Elliptio*	12	25 ± 5	.41 ± .08	.142 ± .043
3. Lanceolate *Elliptio*	9	18 ± 5	.28 ± .11	.094 ± .044
4. *Uniomerus* (3 species)	3	10 ± 8	.29 ± .12	.105 ± .017
5. *Fusconaia flava*	1	16	.29 –	.107 –
6. *"F." succissa*	1	25	.429 –	.081 –
Amblemini				
7. Mb, Qq, Qi, Esl (4 genera, 4 species)	4	24 ± 5	.25 ± .09	.075 ± .038
Lampsilini				
8. Lt[3] (Davis, this paper)	1	25	.29 –	.056 –
9. *L. radiata*	3	20	.305 ± .132	.038 ± .023
10. *L. ochracea*	3	20	.262 ± .040	.043 ± .009
11. *L. splendida*	1	20	.428 –	.059 –
12. *L.* sp. (L. Waccamaw)	1	20	.500 –	.039 –
13. *L. siliquoidea*	1	20	.600 –	.113 –
The 6 species of *Lampsilis*: data, in part: Kat (1983c); Kat, unpublished data. Kat used 14 loci used here.			.397 ± .135	.058 ± .028
Anodontinae				
14. *Anodonta cataracta* Davis *et al.* (1981)	5	23 ± 8	.113 ± .039	.028 ± .006
15. *A. gibbosa*	1	20	.285 –	.106 –
16. *A. implicata*	3	20	.357 ± .000	.061 ± .007
18. *A. fragilis* Kat (1983a)	3	20	.237 ± .041	.081 ± .004
19. *A. grandis* (Kat, personal communication)	1	20	.500 –	.192 –
The five species of *Anodonta*:			.298 ± .143	.094 ± .062
Margaritiferinae				
20. Davis *et al.* (1981)	1	21	.14 –	.030 –

RESULTS

Shells of the lanceolate taxa examined here are shown in Fig. 1. Only five of nine taxa are given names here: *Elliptio lanceolata* (topotype), *E. folliculata, E. producta, E. fisheriana,* and *E. shepardiana.* Shells of species of non-lanceolate, ovate-quadrate *Elliptio* are shown in Fig. 2 along with the triangular shell of *Fusconaia flava.* Shells of the three species of *Uniomerus,* as well as *Lampsilis teres, Quincuncina infucata,* and *Quadrula quadrula* are illustrated in Davis (1983).

The frequency data for 102 alleles × 39 populations are too voluminous to publish here. They are available on request. Nei's I and D are given in Table 2. Average individual heterozygosity (H), index of polymorphic loci (P), and number of individuals used from each of the 39 populations are given in Table 3. Considering all populations, 71.4% of the 14 loci are polymorphic. Selected groups of

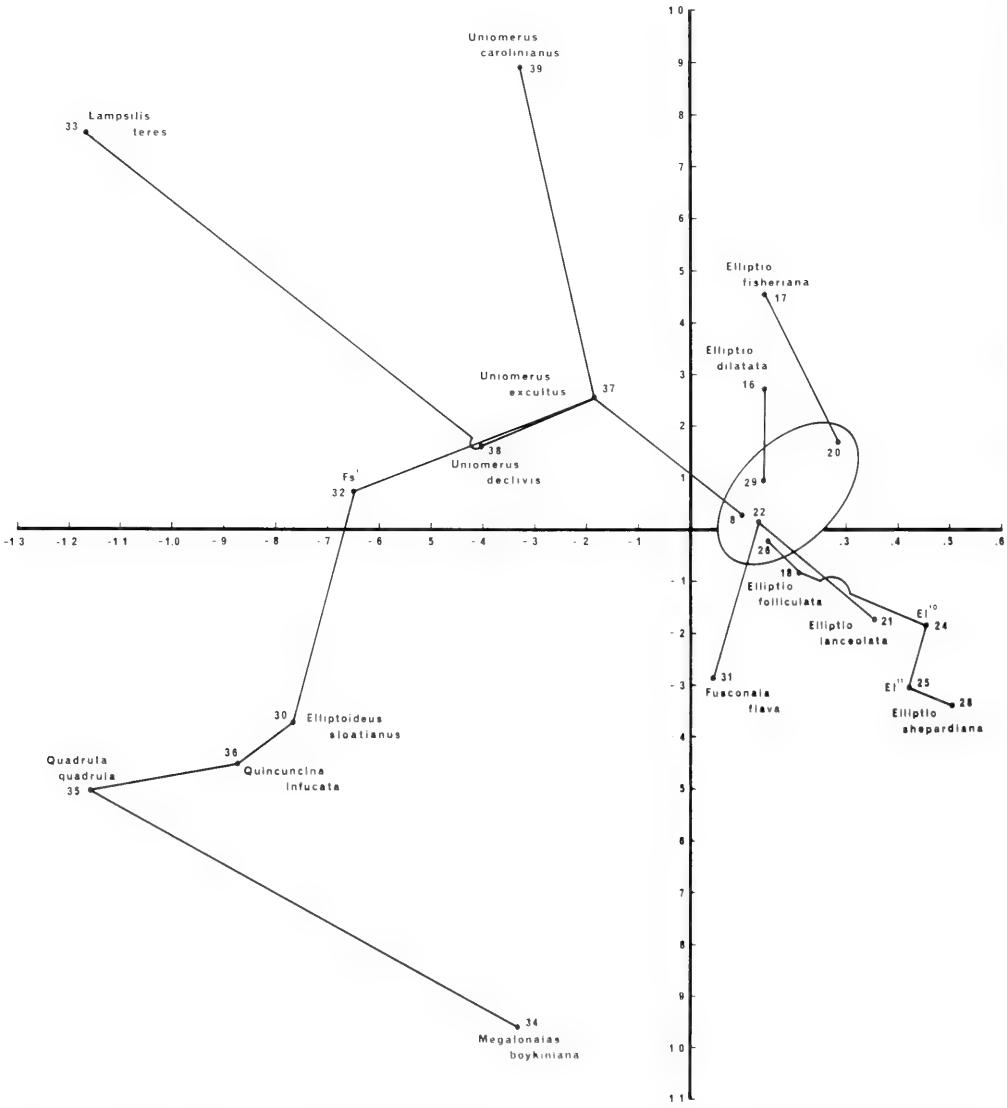

FIG. 3. Ordination diagram of first two axes following 3-D scaling. The Prim Network is used. The ellipse to the right is hand-drawn; it encloses taxa shown in Fig. 4. See text for details.

taxa are listed in Table 4 along with group means and standard deviation of P and H, and sample size per population.

The ordination diagram with Prim Network involving axes I, II, following 3-dimensional scaling based on Nei's statistic is given in Fig. 3. The matrix correlation is 0.951 and 78% of the variance is included. The stress was 0.192 after 50 iterations. The ellipse (drawn by hand) including taxa 20, 29, 22, 26, and 8 is enlarged in Fig. 4 to show clearly the interrelationships of the 22 taxa contained therein. Taxa 20, 29 etc. within the ellipse are shown because they are the closest along the network to taxa outside the ellipse. Finally, in Fig. 5 is given the Prim Network showing the relative distances between taxa of *Elliptio*. Fig. 5 makes it easier to visualize the relationships shown in Fig. 4. Fig. 5 differs from Fig. 4 by showing relationships along the Prim Network freed from the contortions and overlapping lines caused by ordination.

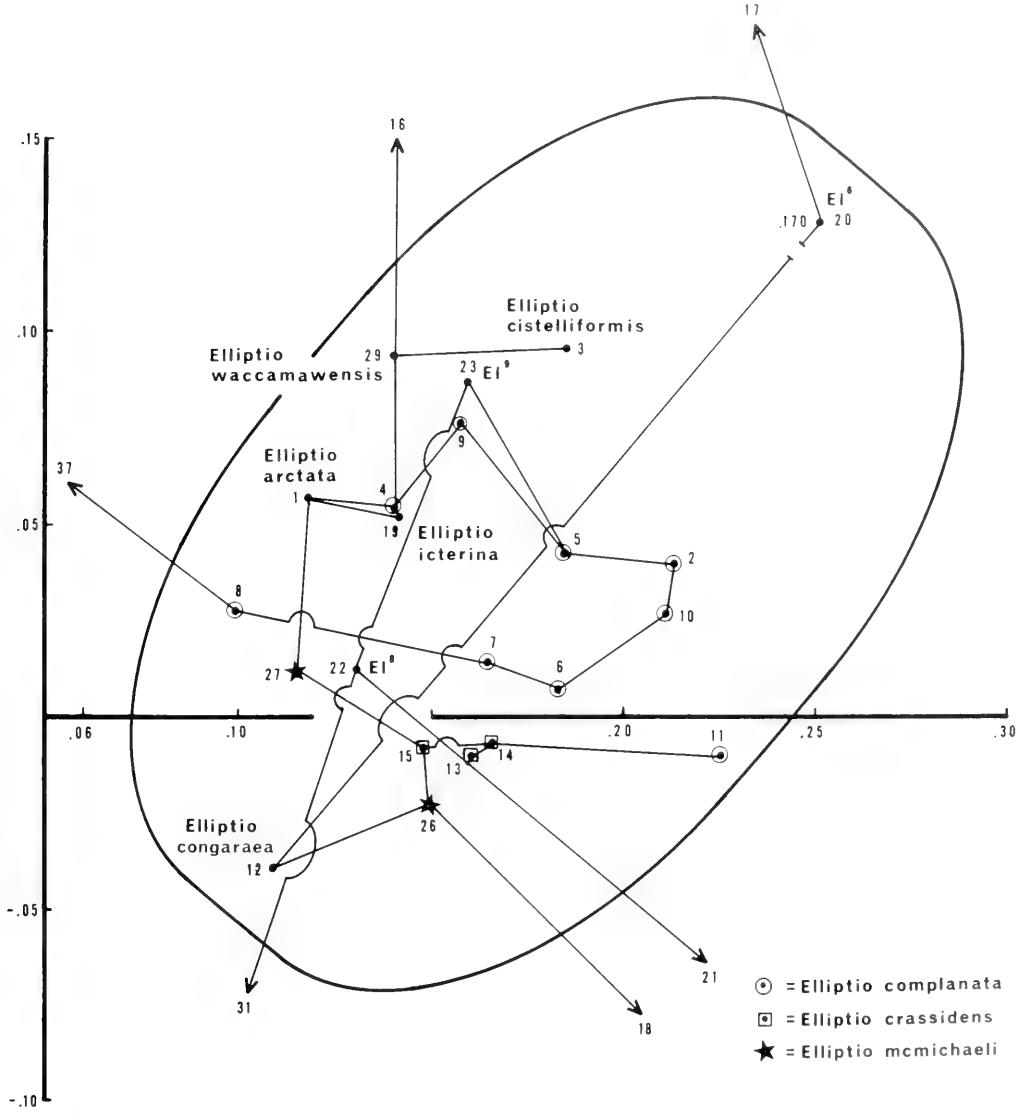

FIG. 4. Enlargement of area in Fig. 3 enclosed by the ellipse. Most ovate-quadrate species of *Elliptio* are within this area.

The following relationships are evident: 1) *Uniomerus* and *Elliptio* are distinct genera, divergent along the Prim network in Fig. 3 with species of *Elliptio* to the right of the vertical axis, and species of *Uniomerus* to the left of the axis. The closest species of the two genera along the Prim network are 79.3% similar (D = 0.232) demonstrating the close genetic relationship between the two genera. The greatest distance along the Prim Network between species of *Uniomerus* and between species of *Elliptio* respecively is 0.484 (I = 0.616) and 0.732 (I = 0.481). Only seven comparisons between species of *Elliptio* yield D ⩾ 0.484, the greatest D between species of *Uniomerus* (Table 5). All of these involve lanceolate taxa of *Elliptio*. 2) *Elliptoideus* and *Quincuncina* are closely allied with *Quadrula* and *Megalonaias* along a network divergent from the *Uniomerus-Elliptio* grouping. 3) *Fusconaia succissa* (Fs, No. 32) is so divergent from *Fusconaia flava* that the two taxa cannot be congeneric (D = 0.468).

Additional significant findings are evident by assessing the relationships among species of *Elliptio* (Figs. 4, 5). There are three divergent lineages of lanceolate *Elliptio*. In all there are at least seven species: lineage I, *E. producta* (El^6, No. 20), and *E. fisheriana* (No. 17); lineage II, *E. lanceolata* (topotypes, No. 21), *E.* spp.? (El, No. 23, El, No. 22); lineage III, *E. folliculata* (No. 18), *E. shepardiana* (No. 28) and *E.* spp. ? (El^{11}, No. 25; El^{10}, No. 24). The three lineages connect to different spe-

TABLE 5. Genetic distances between taxa of *Elliptio* where D ⩾ .484 the greatest distance between species of *Uniomerus*. There were only seven such distances (see text for details). Codes are explained in Table 1. * = lanceolate taxa.

	El^{7*} (21)	El^{8*} (22)	El^{10*} (24)	El^{11*} (25)	Es^{2} (28)
Eco 12	.499	—	—	—	—
Ed 16	—	.532	—	—	—
Efi* 17	.501	.528	.591	.699	.732

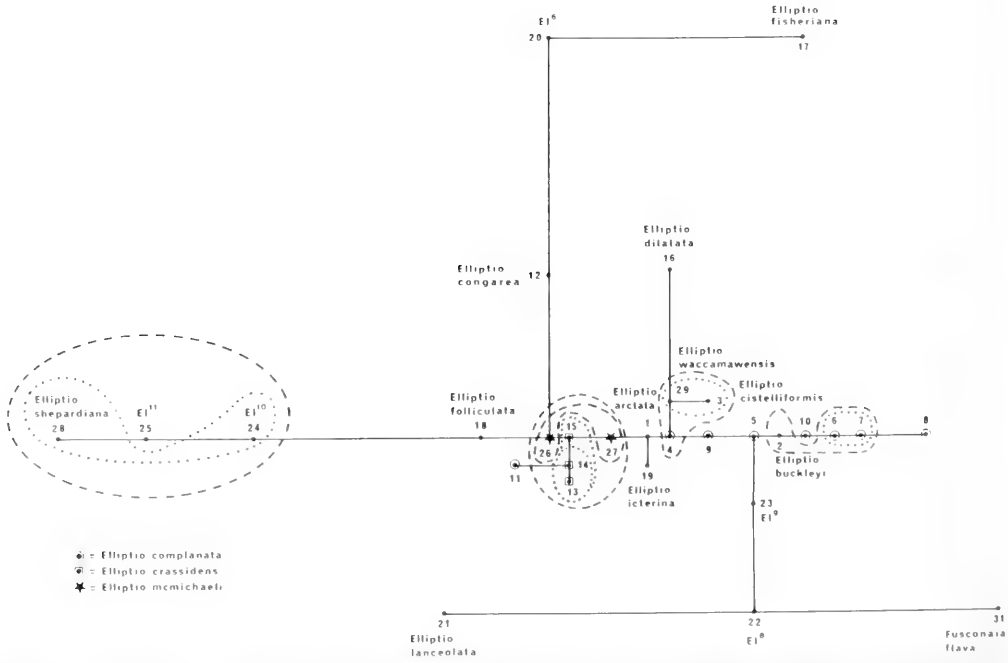

FIG. 5. Relationships among species of *Elliptio* shown by use of the Prim Network (minimum spanning tree) with distances among taxa proportional to those in Fig. 4. Computer-derived sets and subsets are shown as dashed and dotted lines. See text for details.

cies along the Prim Network; lineage I from *E. congaraea* (No. 12); II from *E. complanata* (Ec^{8}, No. 5); III from *E. mcmichaeli* (Nos. 26, 27). I consider these lineages to be clades as defined by the Prim Network shown in Fig. 5. Later in this paper I discuss these lineages in terms of four groups of taxa. As evident in Fig. 5, *E. folliculata* (No. 18) is considerably separated from the cluster of Nos. 24, 25, and 28 along the same segment of network; I discuss these as two different groups. The closest relationship between two taxa in the original matrix of D values (Table 2) is not always the one seen along the Prim Network where relationships are assessed among all taxa. Therefore, closest taxon relationships among lanceolate *Elliptio* taxa as derived from Table 2 are given in Table 6. Also, in examining relationships here it must be understood that addition of data from other nominal species of *Elliptio* to this data base may change relationships discussed here. However, this study involves at least 17 species of *Elliptio*; the most recent synthesis of the genus involving southern Atlantic Slope *Elliptio* (Johnson, 1970) and Interior Basin *Elliptio* results in 15 nominal species and subspecies being considered valid of which only five are not studied here. Lineage I affinities are with *Elliptio waccamawensis* from North Carolina, *E. mcmichaeli,* and *E. arctata* from panhandle Florida, and *E. complanata* of the Delmarva (Delaware-Maryland-Virginia) peninsula. Lineage II affinities are with various *E. complanata* and peninsular Floridian *E. buckleyi.* Lineage III affinities are with *E. mcmichaeli* and *E. crassidens,* panhandle Floridian species.

So-called *Elliptio complanata* from the Savannah River, Georgia (Ec^{28}, No. 11) is not *E. complanata.* Its closest relationships are with populations of *E. mcmichaeli* (D = 0.098, 0.088). Genetic distance between the seven populations of *E. complanata* (excluding population No. 11) ranges from 0.023 to 0.083 (I of 0.977 to 0.920). Population No. 11 from the Savannah River differs from populations Nos. 4–10 of *E. complanata* by a mean D of 0.114 ± 0.013 (I = 0.893 ± 0.012). Population No. 11 is closely related to only one population of *E. complanata* thus far studied, No. 4 from Delmarva with D = 0.099. In summary, the overall affinities of population No. 11 are with *E. mcmichaeli* and *E. crassidens,* species also closely related to population No. 4 of *E. complanata* (D, $\bar{X}$ = 0.075 ± 0.011; N = 5).

Elliptio mcmichaeli and *E. crassidens* are closely related genetically ($\bar{X}$ D of 0.035 ± 0.003; $\bar{X}$ I of 0.965 ± 0.003). *E. dilatata* is divergent from *E. complanata* ($\bar{X}$ D of 0.176 ± 0.31; $\bar{X}$ I of 0.839 ± 0.027).

DISCUSSION

Elliptoideus, Quincuncina and higher classification

The data presented here are consistent with previous results based on immunology and allozyme analyses, as well as a synthesis of morphology and immunology in that the same divergent clades are clearly identified (Davis & Fuller, 1981; Davis *et al.,* 1981; Davis, 1983). These clades have been defined as tribes within the Ambleminae as follows (with genera assigned): Lampsilini

TABLE 6. Closest taxon relationships for each of the nine lanceolate taxa of *Elliptio* based on D values in Table 2. See Fig. 5.

Lineage	Taxon	Closest relationships (D values)			
		1st		2nd	
I	*E. fisheriana* (no. 17)	El^{6} (no. 20)	.222	Ew (no. 29)	.264
	E. producta (no. 20)	Em^{2} (no. 27)	.208	Ea^{2} or Ec^{6} (nos. 1,4)	.217
II	El^{9} (no. 23)	Eb^{2} (no. 2)	.095	Ec^{9} or ^{11} (nos. 6,7)	.099
	El^{8} (no. 22)	Ec.^{9} (no. 6)	.040	Ec^{12} (no. 8)	.059
	E. lanceolata (no. 21)	Ec^{23} (no. 10)	.224	Ec^{9} or El^{8} (nos. 6,22)	.242
III	*E. folliculata* (no. 18)	Em^{2} (no. 27)	.086	Ecr^{5} or Em^{1} (nos. 15,26)	.096
	El^{10} (no. 24)	Es^{2} (no. 28)	.062	El^{11} (no. 25)	.077
	El^{11} (no. 25)	El^{10} (no. 24)	.077	Es^{2} (no. 28)	.105
	E. shepardiana (no. 28)	El^{10} (no. 24)	.062	El^{11} (no. 25)	.105

(*Lampsilis*); Pleurobemini (*Elliptio, Fusconaia, Uniomerus*), and Amblemini (*Elliptoideus, Quadrula, Quincuncina, Megalonaias* = *Amblema* [see Davis & Fuller, 1981]).

While *Quincuncina* was previously shown to be closely related to *Quadrula* in the Amblemini (Davis, 1983) (I = 0.725, D = 0.321), this is the first time that such relationships of *Elliptoideus* have been demonstrated. *Elliptoideus* is found only in the Apalachicola and Ochlockonee River drainages flowing through Florida, U.S.A. to the Gulf of Mexico. The genus is monotypic with *E. sloatianus* (Lea) the type. While *Elliptoideus* Frierson (1927) was classified by Heard & Guckert (1971) in the subfamily Ambleminae (as they defined it), the anatomy of this species has been unknown for the most part. Accordingly, its systematic position was uncertain.

We now know a considerable amount about the anatomy of this species (Fuller *et al.*, in prep.). *Elliptoideus* has several primitive features shared by some or all taxa of Margaritiferinae, i.e. perforate interlamellar gill septa, lack of a mantle suture that divides the excurrent mantle aperture into anal and supra-anal sections, arborescent papillae located at the incurrent mantle aperture, tetragenous mode of reproduction and biradial shell sculpture on the disc. Character-states that are derived non-Margaritiferinae are: septa of gills parallel to the gill filaments; continuous septa; papillae along the ventral parts of the margins of the excurrent aperture (in adults). The combination of primitive character-states coupled with perforate septa and sculptured shell, and allozyme data showing that this genus is closely related to *Quincuncina* and *Quadrula* (Fig. 3), indicate classifying *Elliptoideus* in the Amblemini, a predominantly tetragenous group (see Davis & Fuller [1981], table 13).

Generic relationships and genetic distance

Fusconaia—It is clear that *Fusconaia flava* and *F. succissa* are not congeneric. The genetic distance between them is 0.468. The genetic distance 0.468, while considerable, is not sufficient by itself to consider *F. flava* and *F. succissa* to belong to different genera. What is persuasive is that *F. succissa* links to *Uniomerus* along the Prim Network and is, with *Uniomerus,* divergent from *Elliptio* (Fig. 3). *Fusconaia flava* links to an entirely different group of *Elliptio* than does *Uniomerus* (Fig. 5). On genetic data alone "*Fusconaia*" *succissa* is most closely related to *Uniomerus* (D = 0.375) and *Elliptoideus* (D = 0.375); it is quite distantly related to other genera of the Amblemini (D $\geqslant$ 0.471). Accordingly, this taxon is provisionally placed with the Pleurobemini, an arrangement also seeming reasonable considering the *"F." succissa* links to *Uniomerus* and *F. flava* links to *Elliptio* along the Prim Network (Fig. 3). *Fusconaia flava* is probably closely related to *Fusconaia trigonus* (Lea) (= *F. undata trigonus*), the type-species of *Fusconaia,* as discerned from conchological and general anatomical topological features. Accordingly it is probable that *F. succissa* must either be assigned to another existing genus or have one erected for it.

Therefore, this study involves eight genera aside from the outgroup *Lampsilis*. Three questions can be answered, at least tentatively, with empirical data. 1) What genetic distances occur between unionid genera? 2) What variances are there for genetic distances for genera of different tribes? 3) What genetic distances can be expected between tribes within the Unionidae? It is well understood that no value of D can automatically assign species or generic rank (Davis *et al.*, 1981; Davis, 1983). While some species pairs of vertebrates and invertebrates differ by a D $\geqslant$ 0.050 (Davis *et al.*, 1981; Davis, 1983) differences between congeneric species of vertebrates generally exceed 0.40 (Avise, 1976). Mean differences between congeneric species of freshwater bivalves have been 0.210 to 0.609 depending on the clade (Table 8).

Given the data in Tables 7–9 several points are clear: 1) The D between congeneric species is significantly different when dealing with different clades of similar rank. 2) The D between genera within different clades can be significantly different, especially within the Anodontinae and the Ambleminae: Amblemini when compared with the Ambleminae: Pleurobemini (Table 8). 3) Differences between genera of the Pleurobemini are at a level usually attributed to differences between species. 4) The Pleurobemini and Amblemini are more closely related to each other than to the Lampsilini. 5) There is as much difference between genera of the Amblemini as there is between the tribes Amblemini and Pleurobemini. 6) There is much greater variance in D between genera of the Amblemini than between genera of the Pleurobemini.

TABLE 7. Genetic distances between genera of the tribes Pleurobemini and Amblemini. The distance in ( ) is that between the closest two taxa along the Prim Network representing two different genera (Fig. 3). The distance not enclosed in ( ) is the shortest distance between two taxa of two different genera as obtained from the original data matrix (Table 2).

		Pleurobemini				Amblemini			
		1	2	3	4	5	6	7	8
1. *Elliptio*	(1–29)*	—	.205(.232)	.184 (.245)	.399 (.454)	.503 (.613)	.497 (.538)	.581 (.581)	.595 (.976)
2. *Uniomerus*	(37–39)		—	.341 (.341)	.345 (.483)	.478 (.476)	.458 (.476)	.521 (.602)	.781 (.890)
3. *Fusconaia*	(31)			—	.468	.446	.437	.321	1.050
4. *"Fusconaia"*	(32)				—	.375	.417	.590	1.030
5. *Elliptoideus*	(30)					—	.358	.686	.634
6. *Quincuncina*	(36)						—	.321	.969
7. *Quadrula*	(35)							—	.935
8. *Megalonaias*	(34)								—

*Nos. along the Prim Network.

TABLE 8. Mean genetic distances (D) between congeneric species and genera of the same clade of freshwater bivalves.

	Between congeneric species	Between genera	Reference
Unionidae			
Anodontinae			
Anondonta	.457 ± .073 N = 3	1.111 ± .184* N = 2	Kat (1983a)
Ambleminae			
Amblemini	—	.651 ± .275	This paper
Pleurobemini			
Elliptio	.210 ± .017 N = 7	.243 ± .086 N = 3	Davis (1981); this paper
Fusconaia	—	.243 ± .086 N = 3	Davis (1981); this paper
Uniomerus	.308 ± .165 N = 3	.243 ± .086 N = 3	Davis (1981); this paper
Lampsilini			
Lampsilis	.609 ± .478 N = 6	—	Kat (1983c)
Pisidiidae			
Sphaerium	.568 ± .310 N = 4	—	Hornbach *et al.* (1980)

*Considering that *A. implicata* belongs to a different genus than that of *A. gibbosa*, *A. cataracta*, and *A. fragilis*.

The above points are interpreted by invoking the concept that, in general, genetic distances increase with time. If this concept is correct, the genera of the Pleurobemini are more recently evolved than those of the Amblemini, hence the lower genetic distance and lower genetic variance among genera of the Pleurobemini. It has been assumed that low molecular genetic differentiation has been due to recency of divergence and radiation; there has not been sufficient time to accumulate much molecular genetic difference (Gottlieb, 1973; Wilson *et al.*, 1974; Avise *et al.*, 1975; Davis *et al.*, 1981; Kat, 1983c).

Two cladograms are given in Fig. 6 as hypotheses concerning the cladistic relationships among the taxa. Cladogram A is based on immunological distances, and anatomical data; it is consistent with paleontological data and zoogeography. In Fig. 6A

TABLE 9. Mean genetic distance (D) between tribes and subfamilies. Data within ( ) are D values based on Prim Network distances between closest two species of two genera. The analysis does not include "*Fusconaia succissa*." ? = data not available; dummy values used for multivariate analysis.

	P.	Am	L.	An	M
Ambleminae					
Pleurobemini	—	.556 ± .191	.906 ± .145	1.489*	1.377*
Amblemini		—	1.399 ± .132	?	?
Lampsilini			—	1.726*	1.875*
Anodontinae				—	2.051*
Margaritiferinae					—

*From Davis *et al.* (1981); only *Elliptio, Anodonta cataracta, Lampsilis teres,* and *Margaritifera margaritifera* are involved, thus no standard deviations are given.

FIG. 6. Two cladograms as hypotheses of relationships among North American Unionidae. A. Cladogram derived from Davis & Fuller (1981) based on synthesis of immunological data and anatomical data. B. Cladogram modified from A by additional use of allozyme data using distances derived by NTSYS using two dummy values (see Table 9). The NTSYS program used was MSTSNGL to produce a rectangular tree matrix from which a phenogram was produced (hence the linkage distances).

divergence from proto-*Margaritifera* involved morphological advances, i.e. development of septa and water tubes parallel to the gill filaments, and creation of the diaphragm and supra-anal aperture. This cladogram is also consistent with the relationships among North American Unionacea based on conchiolin layer microstructure (Kat, 1983b). Cladogram B is modified from A through adding allozyme data to the morphological and immunological data. Allozyme data indicate that the Anodontinae diverged from the Margaritiferinae earlier than did the Ambleminae (based on the concept of the greater the genetic distance the greater the time of divergence). Genetic distances are provided to the right of the cladogram. For this scenario to be correct there would have been convergent development in septa and water tubes as well as conchiolin layer microstructural development in lineages giving rise to the Anodontinae and Ambleminae. This could have happened as the Anodontinae have unique tripartite water tubes and developed unique hooks on the glochidia; the Anodontinae have primitive simple conchiolin layers while the Ambleminae largely have differentiated conchiolin layers. In both cladograms divergence began in the late Mesozoic.

Genetic divergence with time as it relates to the Unionidae has been shown for *Elliptio, Fusconaia, Lampsilis, Margaritifera* and *Anodonta* (Davis *et al.*, 1981). Fossil *Margaritifera* and *Anodonta* have been identified in the Cretaceous; both genera are world-wide in the Northern Hemisphere. The Ambleminae stem from the Oligocene and are restricted to North America. Genera of the Pleurobemini and Amblemini are recorded from the Pleistocene or Recent but the fossil record for these taxa is extremely poor. The age estimated for these genera exceeds 10 million years (Davis *et al.*, 1981, fig. 5). Species of the *Elliptio complanata* complex are considered late Pleistocene and Recent and actively radiating today (Davis *et al.*, 1981).

The greatest genetic distances between two species of a genus of Unionidae are

0.484 *Uniomerus* (N = 3), 0.732 *Elliptio* (N = 18+ species), 0.502 for the *Anodonta* group of *A. gibbosa, A. fragilis* and *A. cataracta* (= *Pyganodon*; Kat, 1983a), and 1.227 for *Lampsilis* (Kat, 1983c). If *Lampilis ochracea* belongs to another genus as has been argued (review by Kat, 1983c), then the greatest D among the three species of *Lampsilis* thus far studied would be 0.222. The greatest genetic distance between species of non-lanceolate *Elliptio* is 0.258. The greatest genetic divergence between species of lanceolate *Elliptio* is 0.732. These data give an indication of scope of divergence in different adaptive radiations seen among contemporaneous species of different subfamilies and tribes. However, *Anodonta* is not apparently radiating in the Recent; it is decreasing in diversity. These values frequently exceed the minimal distance between two species of two closely related genera (e.g. between *Elliptio* and *Uniomerus,* D = 0.205). There is as much divergence between species of *Uniomerus* as there is among species of *Pyganodon* and *Elliptio.* This is of interest because until recently it was thought that *Uniomerus* was monotypic (see Davis, 1983). Conchological similarities and variance make it difficult to separate species of *Uniomerus* clearly. However, given the extent of genetic divergence between species of *Uniomerus,* the genus is as genetically diverse as is *Elliptio.*

Lanceolate *Elliptio*

The lanceolate group is loosely defined as those populations having shell $^{H}/_{L} \leq 0.43$. Central to the group as defined by $^{H}/_{L}$ are *E. lanceolata* and *E. shepardiana.* Excluded from the group are *E. arctata* and *E. icterina* where $^{H}/_{L}$ is greater than but close to 0.43. The mean $^{H}/_{L}$ of the nine lanceolate populations of this study is 0.37 ± 0.06. Shell shape is variable as seen in Fig. 1. *E. lanceolata, E. shepardiana, E. fisheriana,* and *E. producta* have the idealized concept of lanceolate shape.

Until recently (Davis *et al.,* 1981) the lanceolate group consisted of only *E. lanceolata* (with 20 synonyms) and *E. shepardiana* (Johnson, 1970). *E. lanceolata* was considered allied to *E. icterina* and *E. complanata* but easily separated from them by having a shell over twice as long as wide, and by having shells with nearly parallel dorsal and ventral margins (Johnson, 1970). *E. fisheriana* was considered an ecophenotype. Johnson further stated that *E. shepardiana* was obviously related to *E. lanceolata.* From this study it is clear that there has been parallel evolution of a lanceolate shape among *Elliptio* (Fig. 5). This is also made clear in Table 10.

The three lineages of *Elliptio* are compared in terms of four groups of taxa and D valves (Table 10). These groups are called *E. shepardiana, F. folliculata, E. fisheriana,* and *E. lanceolata* groups. The *E. shepardiana* group is the most cohesive genetically (D = 0.081 ± 0.022) and zoogeographically. There are three species considering the considerable conchological differences among them as well as the genetic distances and pattern of distribution. Two are from the Altamaha River drainage (Ocmulgee River) and one is from the Savannah River. Their closest relationships are with Floridian species of the panhandle of Florida (*E. crassidens* and *E. mcmichaeli*). As previously noted (Johnson, 1970, pl. 1) the headwaters of the Coosa-Alabama Rivers, Apalachicola River of Florida and the Savannah River of Georgia are so close as to indicate a series of stream captures between systems over time. Also indicated are areas of suspected stream capture between streams of the Coosa-Alabama River and the Apalachicola River, and streams of the Choctawhatchee and Chattahoochee Rivers. The Chattahoochee River is part of the Apalachicola River. The evolution of *E. crassidens, E. mcmichaeli,* and the three species of the *E. shepardiana* complex from a common ancestor in the river systems indicated is highly probable considering a series of stream captures and divergence.

The greatest genetic distance between species of *Elliptio* is between *E. shepardiana* of group I and *E. fisheriana* of group III (Tables 2, 12; D = 0.732). The mean intergroup D = 0.540 is the greatest D between the four lanceolate groups. The considerable genetic distances between taxa of these two groups (I, III) is probably a reflection of geographic distance, age of divergence, and origin from different ancestral stock within *Elliptio. E. fisheriana* is the northernmost lanceolate *Elliptio* and of the taxa sudied here, is most closely related to lanceolate *E.* cf. *producta* from Lake Waccamaw, North Carolina. *E. fisheriana* clearly is not an ecophenotype to be considered synonymous with *E. lanceolata.* The next closest relationships are with *E. waccamawensis* also from Lake Waccamaw and *E. complanata* from Delmarva Peninsula, the type-locality of *E. fisheriana. E.*

TABLE 10. Comparison of lanceolate taxa placed into groups on the basis of clades derived from Fig. 5 and genetic distances. D values are from Table 2.

	Groups								
	I *shepardiana*			II *folliculata*	III *fisheriana*		IV *lanceolata*		
Taxon no.	28	25	24	18	20	17	23	22	21
28	—	.105	.062	.277	.466	.732	.324	.248	.361
25		—	.077	.271	.433	.699	.321	.255	.437
24			—	.180	.320	.591	.274	.251	.371
18				—	.230	.433	.186	.252	.308
20					—	.222	.373	.451	.385
17						—	.475	.528	.501
23							—	.129	.304
22								—	.242
21									—

	I	II	III	IV
I	—	.242 ± .054	.540 ± .161	.316 ± .065
II		—	.332 ± .143	.249 ± .061
III			—	.452 ± .062
IV				—

cf. *producta* has its closest relationships, in this study, with *E. mcmichaeli, E. arctata* and *E. complanata. E. arctata* (no. 1) from panhandle Florida can be considered part of the *E. complanata* complex as D = 0.083 ± 0.024. *E. arctata* is also closely allied with *E. crassidens* (D = 0.082 ± 0.009), less so with *E. mcmichaeli* (D = 0.095 ± 0.012). Adding these facts together it becomes probable that the *E. fisheriana* clade evolved from a section of the *E. complanata* complex involving a direction of evolution from the panhandle Florida–Georgia drainages northward to coastal North Carolina and finally to the Delmarva Peninsula on the east shore of the Chesapeake Bay. Thus far the lanceolate shape in this lineage is associated only with taxa from North Carolina northeastward.

Elliptio folliculata is considered part of the clade involving the *E. shepardiana* group (Fig. 5) but is examined separately as group II in Table 10. It is considered separately because it does not group closely with any of the three widely separated clusters, i.e. groups I, III, and IV. While *E. folliculata* is the northeasternmost lanceolate species of the *E. shepardiana* clade, it is highly divergent from taxa of the *E. fisheriana* clade. This species from Lake Waccamaw, North Carolina has some conchological similarity with its closest relative, El^{10} (no. 24) of the *E. shepardiana* group from the Savannah River, Georgia (D = 0.180). It is closely related to El^{9} (no. 23) of the *E. lanceolata* group. It is so closely related to *E. mcmichaeli* and *E. crassidens* that it is highly probable that it arose from a lineage ancestral to *E. mcmichaeli* and *E. crassidens. E. folliculata* is also closely related to No. 11 (Ec^{28}), the so-called *E. complanata* from the Savannah River (D = 0.109).

I do not consider Ec^{28} to be *E. complanata* but a distinct species closely allied to *E. folliculata.* Ec^{28} (Fig. 2H) is conchologically between *E. complanata* (Fig. 2C) and some lanceolate *Elliptio* (e.g. Fig. 1G, I). The shell H/L of Ec^{28} is 0.46. I am tentatively placing Ec^{28} in the *E. folliculata* group.

The *E. lanceolata* clade (group IV) is more closely related to the *E. shepardiana* clade (groups I, II) than to the *E. fisheriana* clade (Table 10). This group, more than the other lanceolate groups, is closely related to *Elliptio complanata,* especially from the Ogeechee River drainage (Georgia) and the Delmarva Peninsula. One species, El^{9} is closely related to *E. buckleyi* of eastern Florida, and $Ec^{9,11}$ from Delmarva and also from Maine. *E. buckleyi* is a member of the *E. complanata* species group. This clade apparently evolved from ancestors giving rise to southern *E. complanata* that subsequently dispersed northeastward to Delmarva with some populations

becoming established in Maine. While topotypical *E. lanceolata* is from the Tar River of North Carolina the other two taxa of this complex are sympatric in Buckhead Creek of the Ogeechee River in Georgia south of the Savannah River but north of the Altamaha River.

In summary, the number of species of *Elliptio* has been considerably underestimated in recent times primarily due to an uncritical reliance on conchology. It is also seen here that assignment of taxa to a genus on the basis of conchology can be wrong. There has been considerable convergence and parallelism in shell shape in the Unionidae; it is futile to attempt to define species and assess relationships on the basis of shells alone. There are three divergent clades of lanceolate *Elliptio* that are the oldest, most diverse elements of the genus if genetic distance is indeed correlated with time. The clades have apparently evolved from different ancestors within the evolving *Elliptio* radiation. The most extremely lanceolate taxa are the most distantly related (among themselves) of *Elliptio* species, i.e. *E. shepardiana, E. fisheriana, E. lanceolata*. Thus far sympatry of different lanceolate clades occurs only in Lake Waccamaw and the Savannah River. One can see a transition in shell shape from an *E. complanata* type to the lanceolate shape in taxa of the *E. shepardiana* and *E. lanceolata* clades. It is not the purpose of this paper to give names for the lanceolate taxa; this will be done in a later paper.

Heterozygosity and Polymorphism

Data summarizing our knowledge of these parameters for unionids are given in Tables 3 and 4. Values of H and P can be expected to increase significantly with increasing sample size to a point. Sample sizes here are, with the exception of *Uniomerus,* relatively equal with the mean ranging from 18 to 26. We had fewer lanceolate specimens per population than we would have liked; we attempted to obtain at least 25 individuals for each population. With 25 individuals a single "rare" allele would have a frequency of 0.02. Results with 25 ± 8 individuals should provide an adequate estimate of at least parameter H. Given the limitations of sample size and excluding *Uniomerus* from consideration because the low sample size of three and five individuals in two of the three populations, some trends are clear. The highest levels of P and H are associated with non-lanceolate *Elliptio*. However, the Ec^{28}, which has a shell shape between the *E complanata* type and lanceolate, and which I place in the lanceolate *E folliculata* group, has the highest H of any unionid population thus far studied, i.e. 0.214.

It was previously considered probable that high genetic diversity (high values of H and P) was associated with an active adaptive radiation involving speciation and high interpopulation shell phenotypic variability such as seen in the *E. complanata* complex. With the data available at that time low genetic diversity appeared associated with declining species diversity, ancient lineages and low shell phenotypic variability such as seen in the Anodontinae and Margaritiferinae (Davis *et al.,* 1981). With much more data the above scenario is not that simple. Data for five species of *Anodonta* indicate that, as a group, *Anodonta* is on a par with non-lanceolate *Elliptio* in terms of H and P; two species (40%) have $H > 0.10$. *Anodonta grandis* has considerable H, i.e. 0.19. However, as a group, *Anodonta* is one with lower genetic diversity compared with the non-lanceolate group of *Elliptio* where nine species (90%) have $H > 0.10$.

Lampsilis is considered a highly successful radiation with more than 20 species. The genus is endemic to North America, is among the most specialized of the Unionidae (considering anatomical character-states) and exhibits moderate interpopulation shell phenotypic variability. However, H averages 0.058; only one species (16.6%) has $H > 0.10$ (Kat, 1983c). Thus far we see lower values of H and P among species of *Lampsilis* than we do among species of *Anodonta*.

In summary, it still appears that the greatest genetic diversity is associated with the most phenotypically diverse and actively radiating group today, the non-lanceolate *Elliptio* group. Levels of H and P are not correlated with numbers of species within a genus, i.e. they are not correlated with the size of an adaptive radiation. There is an overall decrease in P and H in older lineages such as the Margaritiferinae, Anodontinae, and Ambleminae: Lampsilini. However, some species of older lineages may have high levels of H and P.

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LITERATURE CITED

AVISE, J. C., 1976, Genetic differentiation during speciation. In: *Molecular Evolution,* AYALA, F. J., ed. Sinauer Associates, Sunderland, Mass., U.S.A., p. 106–122.

AVISE, J. C., SMITH, J. J. & AYALA, F. J., 1975, Adaptive differentiation with little genetic change between two native California minnows. *Evolution,* 29: 411–426.

BURCH, J. B., 1973, Freshwater unionacean clams (Mollusca: Pelecypoda) of North America. United States Environmental Protection Agency. *Biota of Freshwater Ecosystems; Identification Manual* 11, 176 p.

BURCH, J. B., 1975, *Freshwater unionacean clams (Mollusca: Pelecypoda) of North America.* Revised Ed. Malacological Publications, Hamburg, Michigan, 204 p.

DAVIS, G. M., 1981a, Introduction to the second international symposium on evolution and adaptive radiation of Mollusca. *Malacologia,* 21: 1–4.

DAVIS, G. M., 1981b, Different modes of evolution and adaptive radiation in the Pomatiopsidae (Prosobranchia: Mesogastropoda). *Malacologia,* 21: 209–262.

DAVIS, G. M., 1983, Relative roles of molecular genetics, anatomy, morphometrics and ecology in assessing relationships among North American Unionidae (Bivalvia). Systematics Association Special Volume No. 24, *Protein Polymorphism: Adaptive and Taxonomic Significance,* OXFORD, G. S. & ROLLINSON, D., ed. Academic Press, London, p. 193–222.

DAVIS, G. M. & FULLER, S. L. H., 1981, Genetic relationships among Recent Unionacea (Bivalvia) of North America. *Malacologia,* 20: 217–253.

DAVIS, G. M., HEARD, W. H., FULLER, S. L. H. & HESTERMAN, C., 1981, Molluscan genetics and speciation in *Elliptio* and its relationship to other taxa of North American Unionidae (Bivalvia). *Biological Journal of the Linnean Society,* 15: 131–150.

FRIERSON, L. S., 1927, *A classified and annotated check list of the North American Naiades.* Baylor University Press, Waco. 111 p.

FULLER, S. L. H., BEREZA, D. J., DAVIS, G. M. & VIDRINE, M., in preparation, A natural history of the ancient washboard, *Elliptoideus sloatianus* (Lea) (Mollusca: Bivalvia: Unionidae) of the eastern Gulf of Mexico drainage of the United States.

GOTTLIEB, L. B., 1973, Enzyme differentiation and phylogeny of *Clarkia franciscana, C. rubicunda,* and *C. amoena. Evolution,* 27: 205–214.

HEARD, W. H. & GUCKERT, R. H., 1971 ["1970"], A re-evaluation of the Recent Unionacea (Pelecypoda) of North America. *Malacologia,* 10: 333–355.

HORNBACH, D. J., McLEOD, M. J., GUTTMAN, S. I. & SEILKOP, S. K., 1980, Genetic and morphological variation in the freshwater clam, *Sphaerium* (Bivalvia: Sphaeriidae). *Journal of Molluscan Studies,* 46: 158–170.

JOHNSON, R. I., 1970, The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southern Atlantic slope region. *Bulletin of the Museum of Comparative Zoology (Harvard),* 140: 263–450.

KAT, P. W., 1983a, Genetic and morphological divergence among nominal species of North American *Anodonta* (Bivalvia: Unionidae). *Malacologia,* 23: 361–374.

KAT, P. W., 1983b, Conchiolin layers among the Unionidae and Margaritiferidae (Bivalvia): microstructural characteristics and taxonomic implications. *Malacologia,* 24: 298–311.

KAT, P. W., 1983c, Genetics, morphological divergence, and speciation among *Lampsilis. Journal of Molluscan Studies,* 49: 133–145.

KAT, P. W., 1983d, Patterns of electrophoretic and molecular variability in a widely distributed unionid: an initial survey. *Netherlands Journal of Zoology,* 33: 21–40.

KAT, P. W., 1984, Parasitism and the Unionacea (Bivalvia). *Biological Review,* 59: in press.

KAT, P. W. & DAVIS, G. M., in press, Genetic divergence of peripheral populations of Nova Scotian Unionidae. *Biological Journal of the Linnean Society.*

NEI, M., 1972, Genetic distance between populations. *American Naturalist,* 106: 283–292.

WILSON, A. C., SARICH, V. M. & MAXSON, L. R., 1974, The importance of gene rearrangement in evolution: evidence from studies on rates of chromosomal, protein, and anatomical evolution. *Proceedings of the National Academy of Sciences* [U.S.A.] 71: 3028–3030.

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An asterisk (*) denotes a new taxon

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